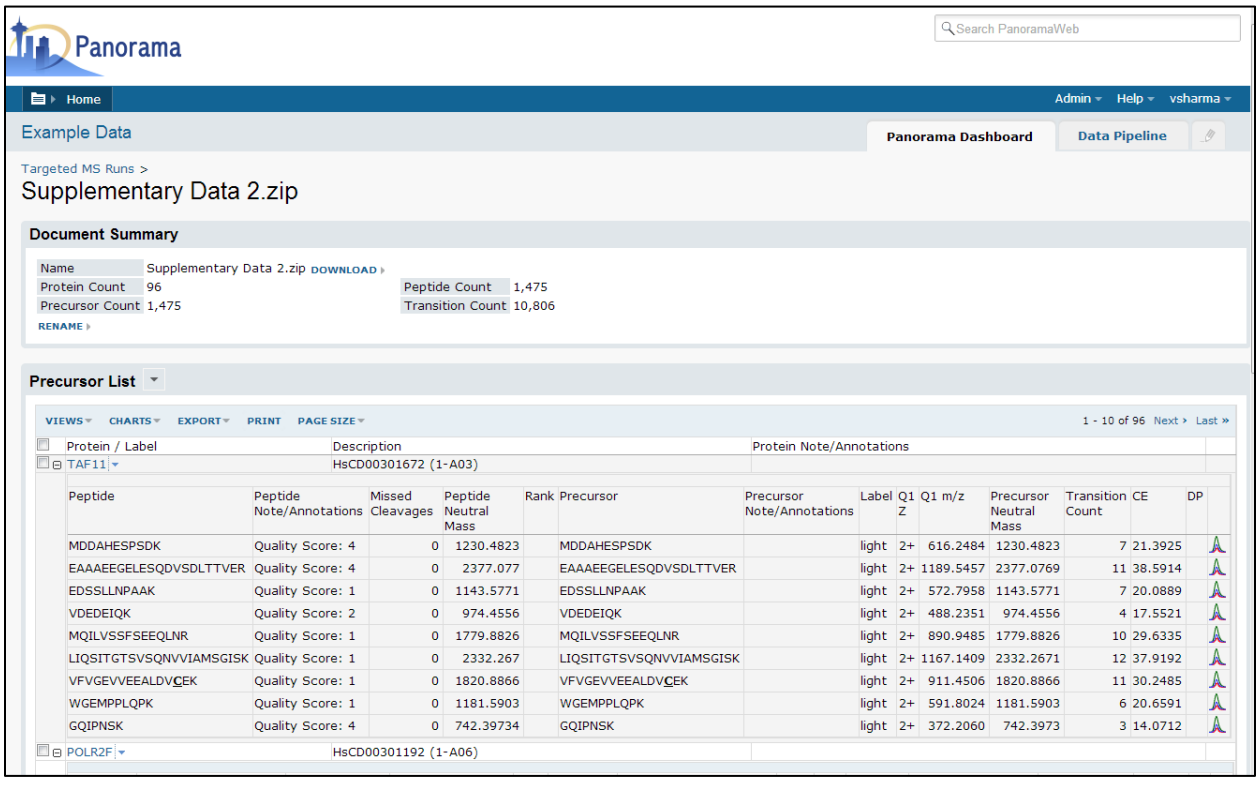
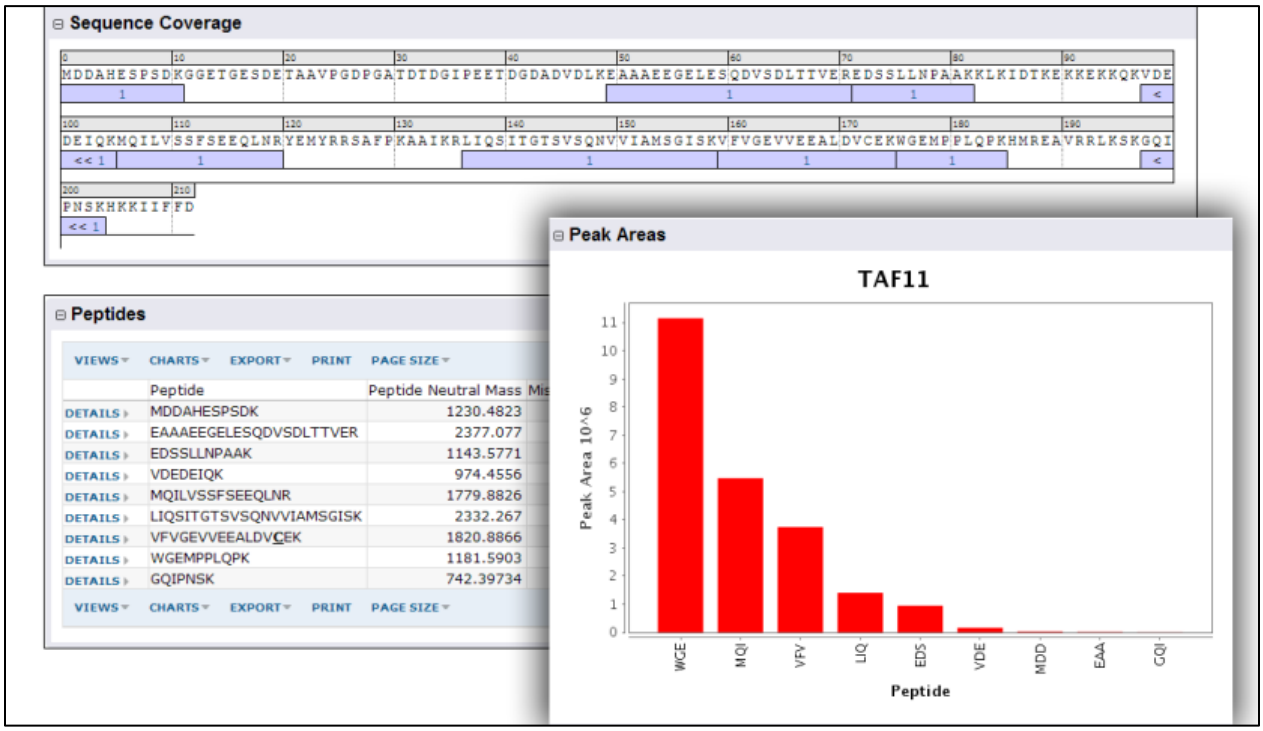
Panorama: A Targeted Proteomics Knowledge Base.

The latest versions of the tutorials are posted at:
https://panoramaweb.org/labkey/wiki/home/page.view?name=tutorials

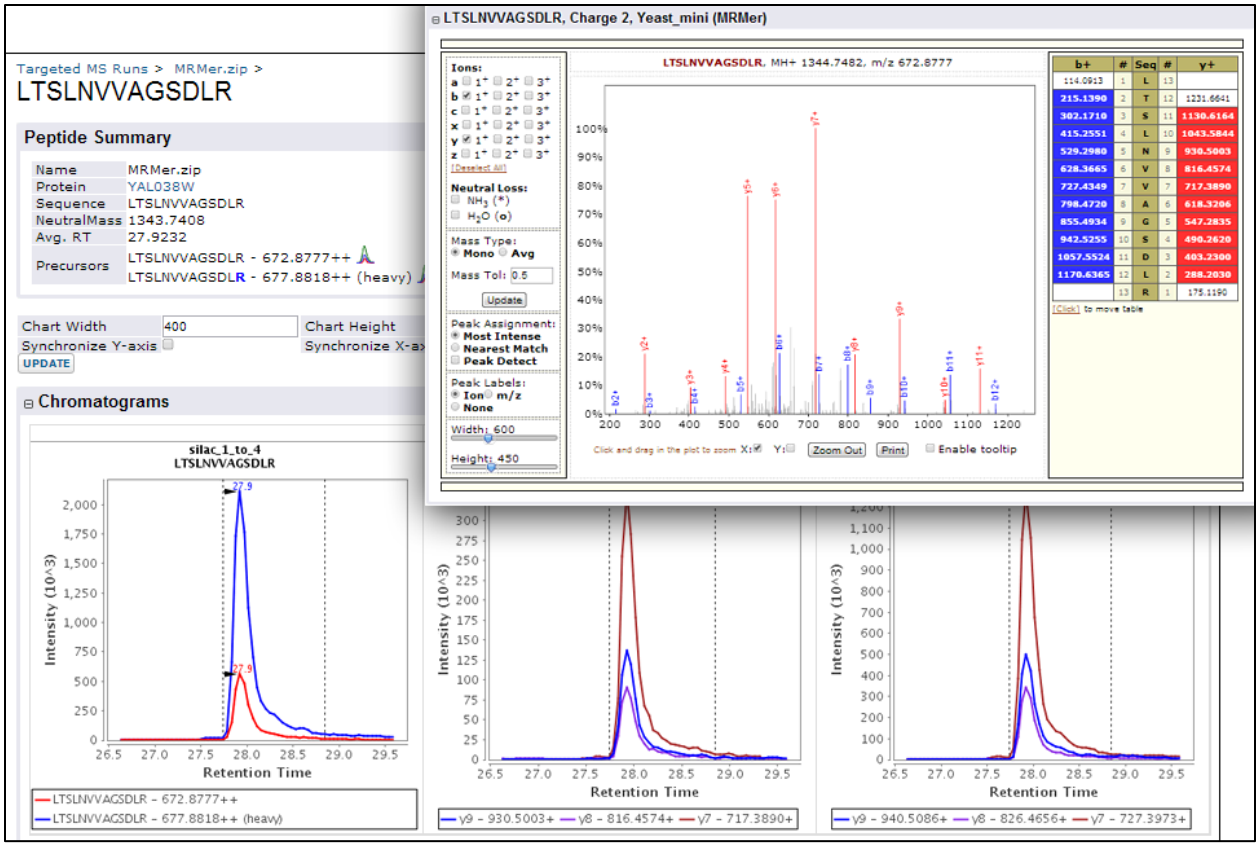


Supplementary Figure 1. The document details view displays a list of the proteins and peptides in the document. Users can click on a protein name or peptide sequence to view more details. The "Quality Score" values in the "Peptide Note/Annotations" column are custom peptide annotations, assigned in Skyline by the author of this

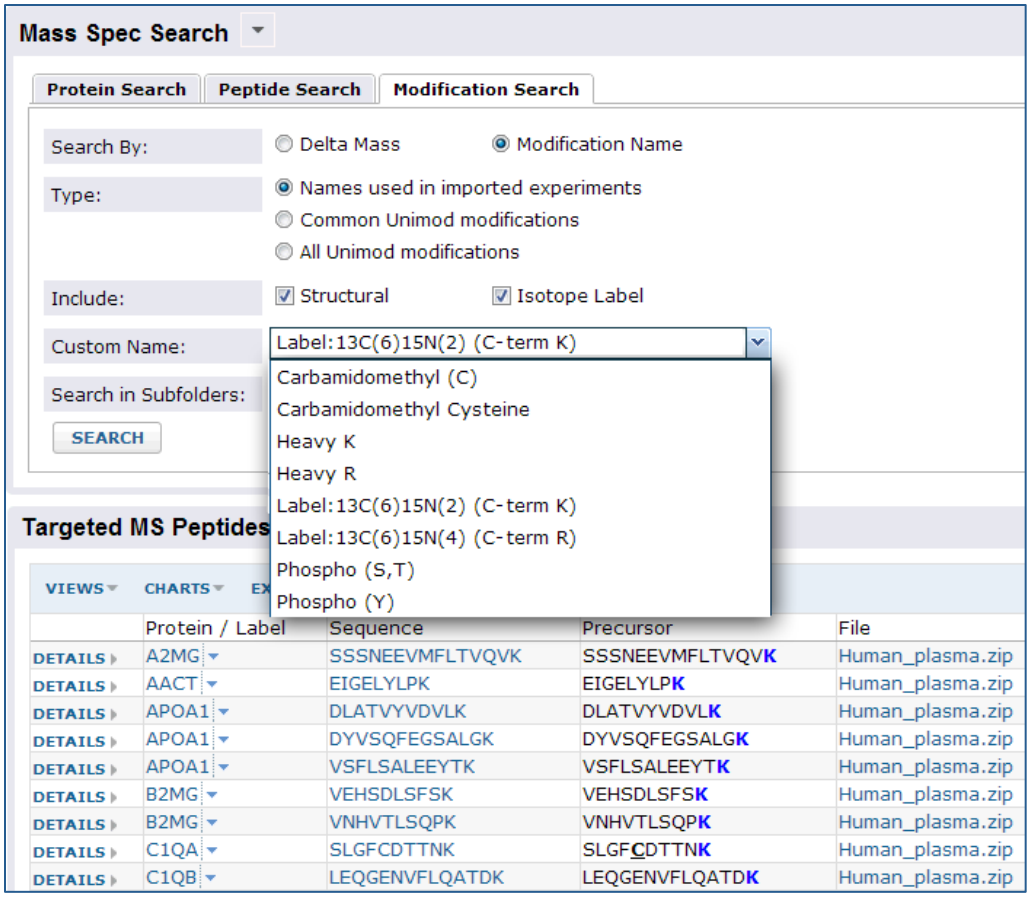
document, based on visual inspection of the chromatograms. Skyline supports manual curation of data via custom, user-defined annotations on the various elements in a document (replicates, proteins, peptides, transitions etc.)



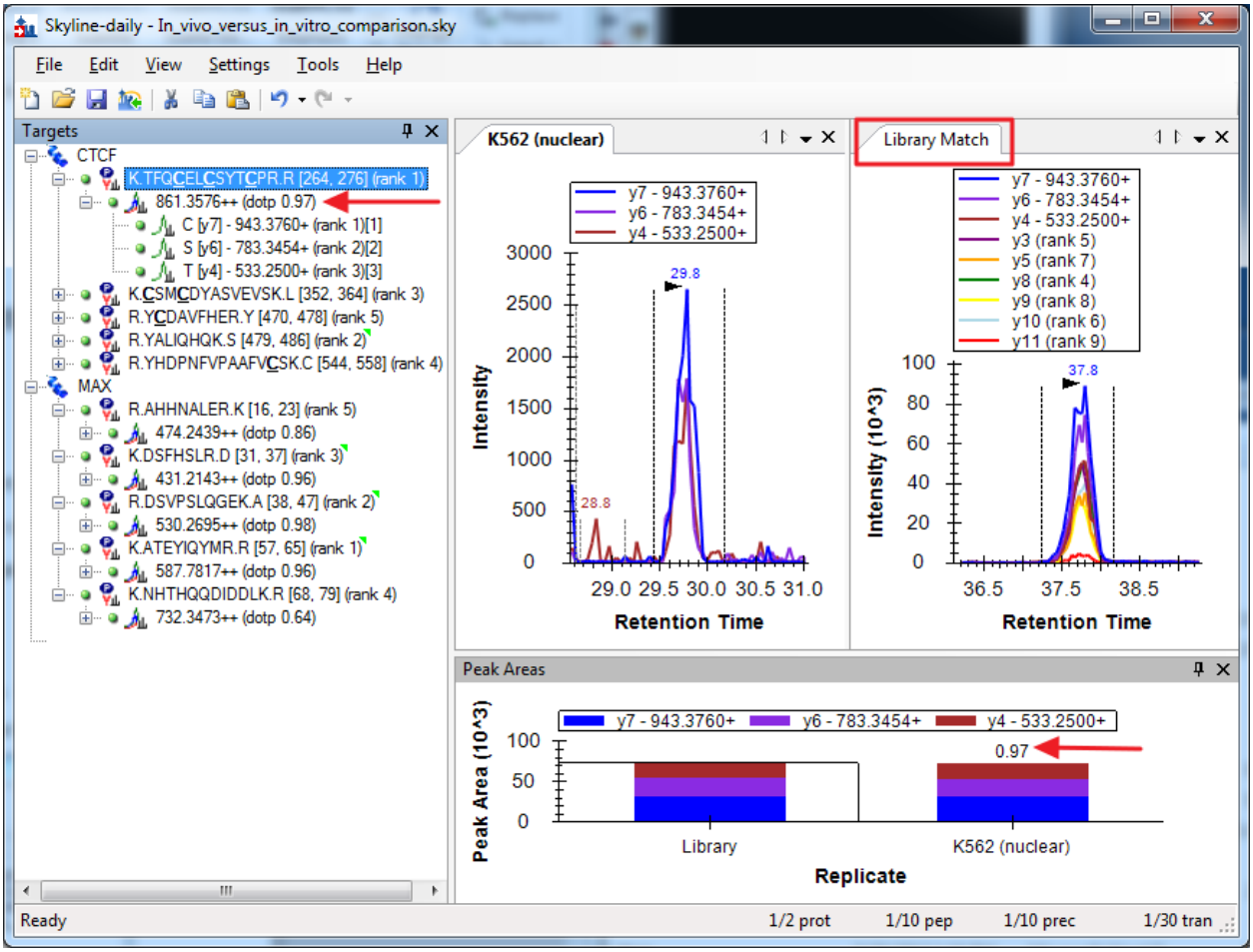
Supplementary Figure 2. The protein details view displays the protein sequence coverage and other information for all the matching peptides. A bar graph displays the peak areas of the peptides ordered from the highest to the lowest measured peak area.



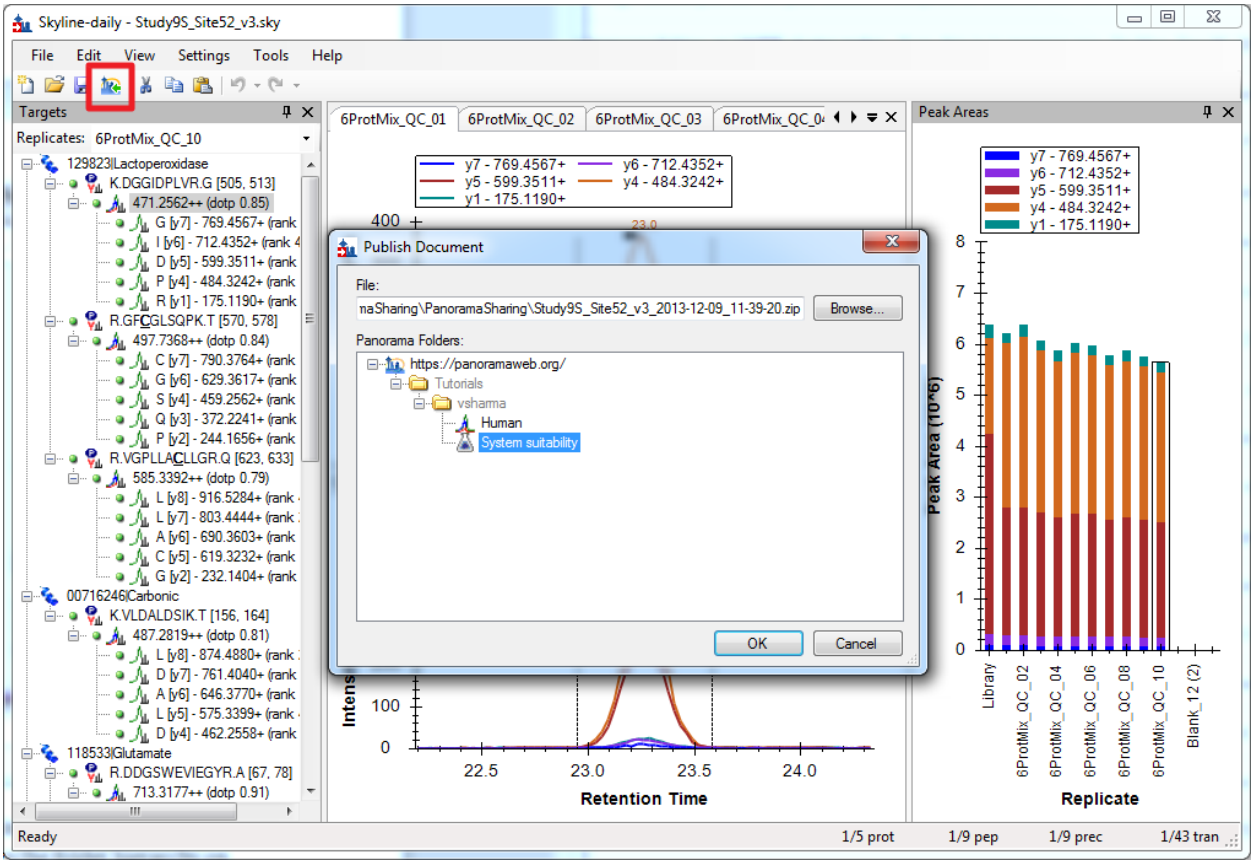
Supplementary Figure 3. The peptide details view displays chromatograms for the peptide in each replicate. Precursor and transition chromatograms are displayed side by side for easy reference. Where available, a library spectrum of the peptide is also displayed.



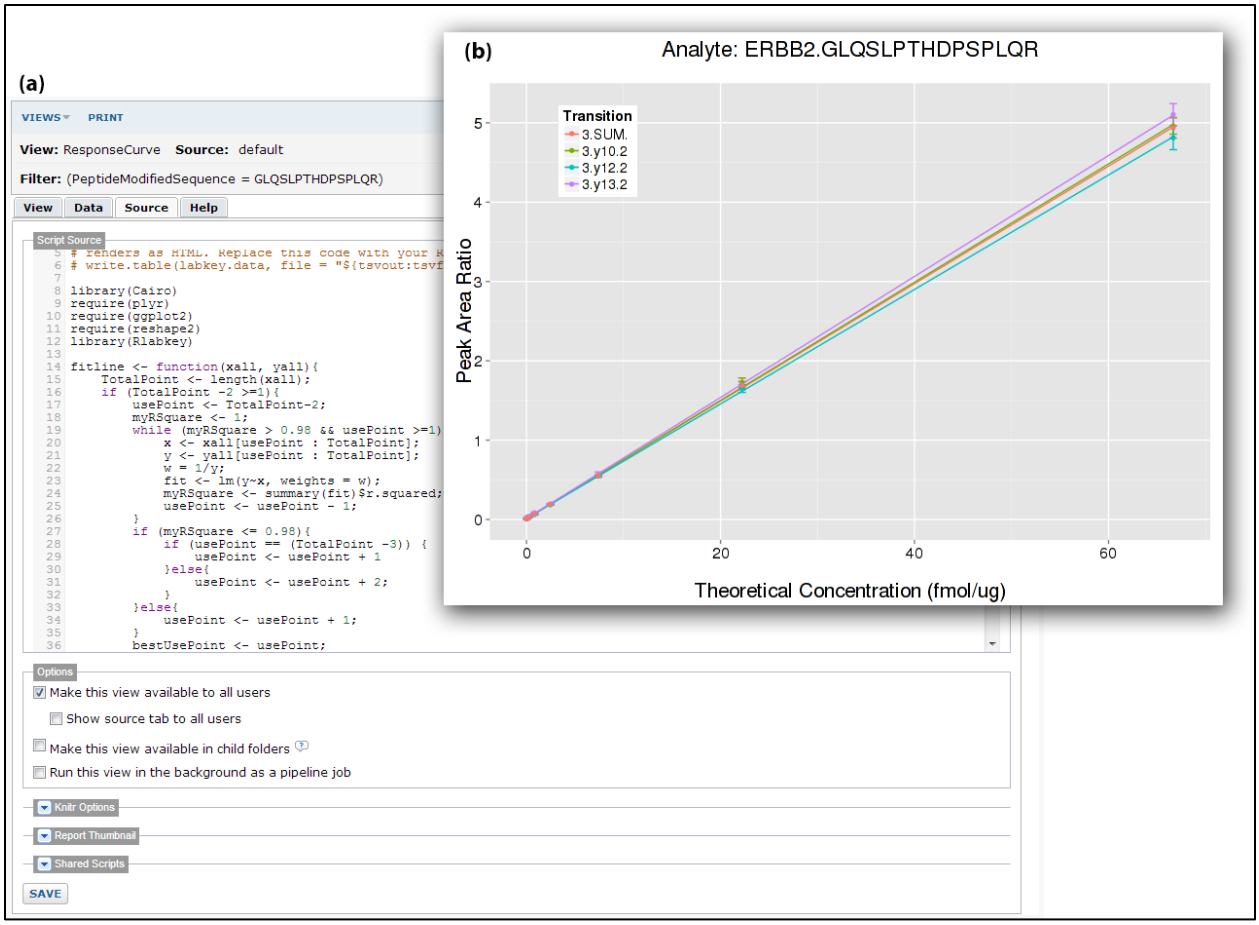
Supplementary Figure 4. The search interface in Panorama allows users to search by protein name, peptide sequence or modified peptides, where the modification may be specified either by name or delta mass. Documents imported to subfolders of the current folder may also be searched.



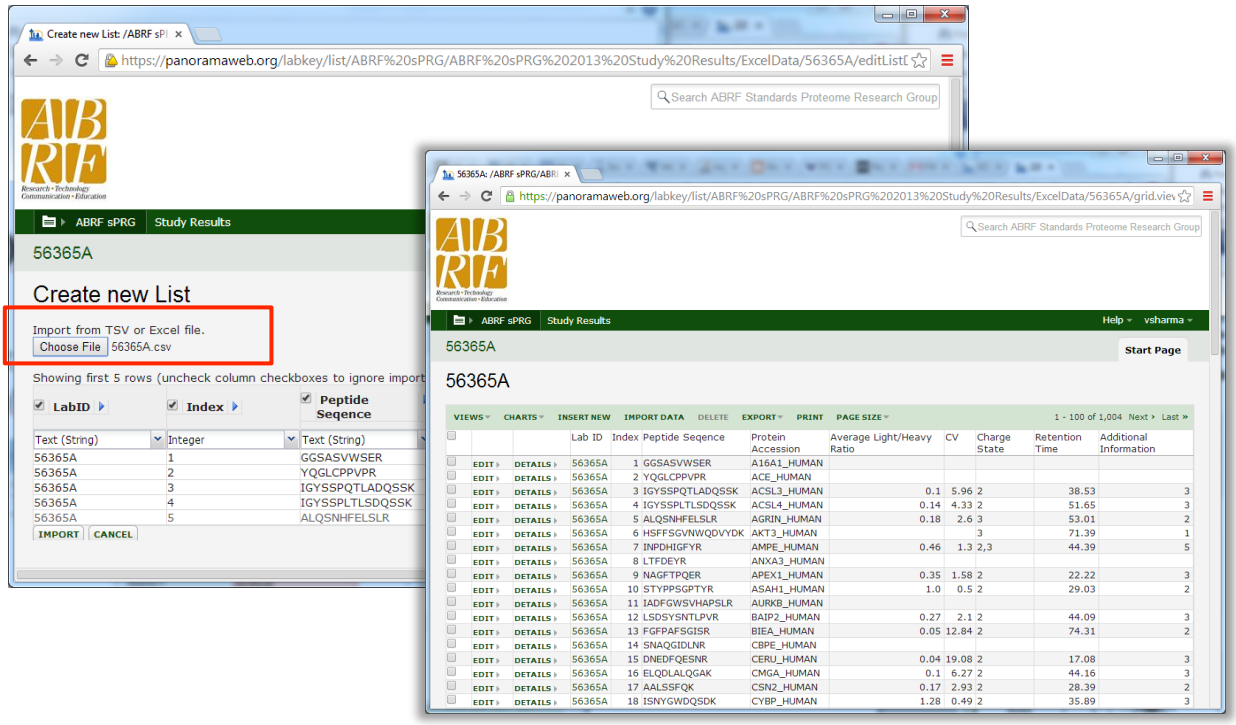
Supplementary Figure 5. Reference chromatograms from a library can be compared with newly acquired data. The tab titled **K562 (nuclear)** shows the chromatogram from the new data, and the tab titled **Library Match** displays the library chromatogram for the peptide **TFQCELCSYTCPR**. Skyline calculates a dot product as a measure of similarity between the new data and the library chromatogram. This dot product is displayed next to the precursor as dotp and also in the peak areas graph, as marked in the image above. For the peptide **TFQCELCSYTCPR** the similarity is high with a dot product of 0.97, and that gives a measure of confidence in the peptide identification.



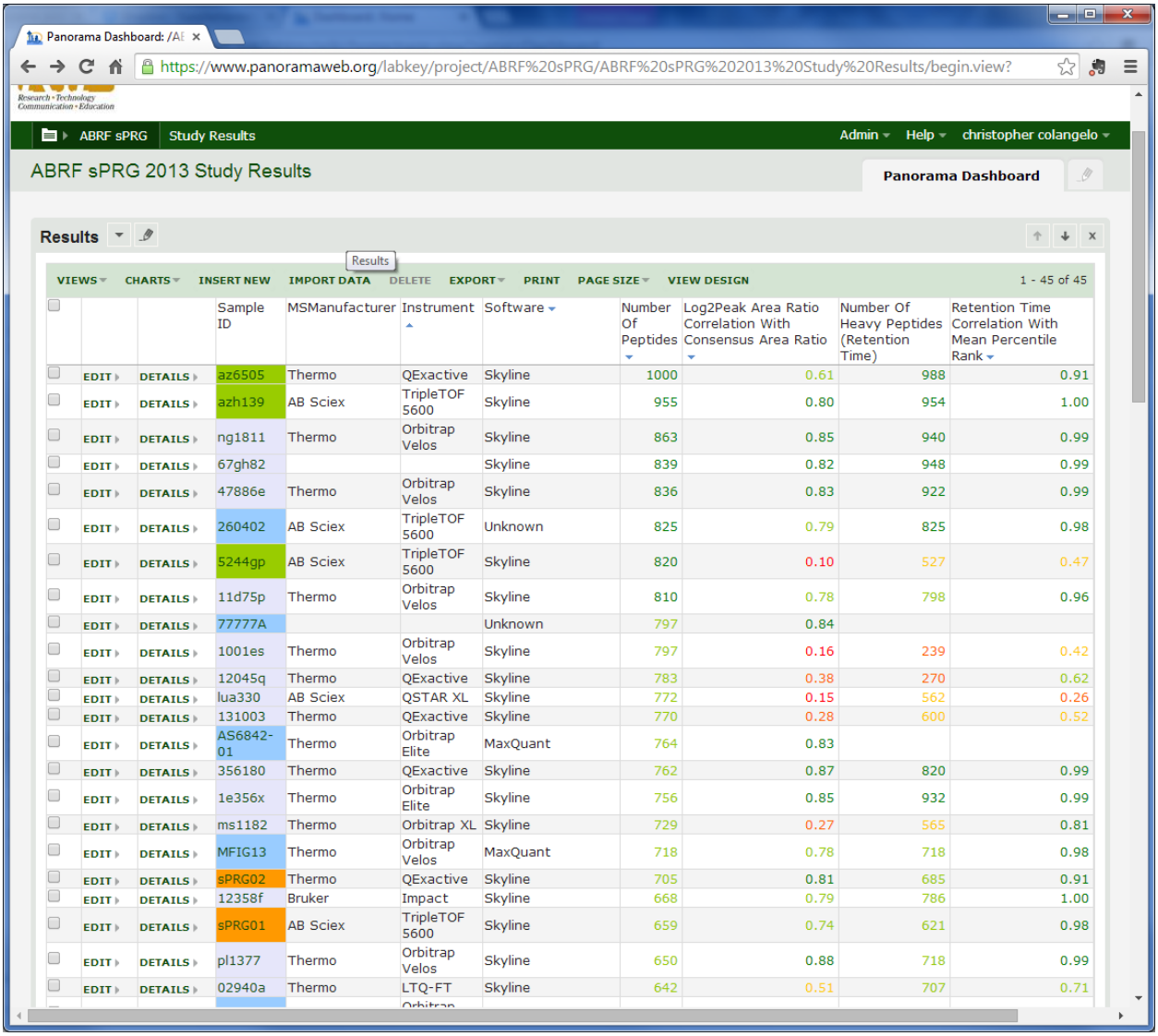
Supplementary Figure 6. Users can publish their documents to Panorama simply by clicking on the "Publish to Panorama" button, highlighted in the figure above. The "Publish Document" form displays the folder hierarchy on the Panorama server and allows the user to select a folder where the document should be uploaded. Only folders to which the user has access are displayed.



Supplementary Figure 7. The R view builder in LabKey Server lets users write custom scripts in the R statistical programming language to analyze available data and display image or tabular output. **(a).** R script written by CPTAC members that retrieves data published to Panorama, for the CPTAC assay portal, to generate a response curve **(b)**.



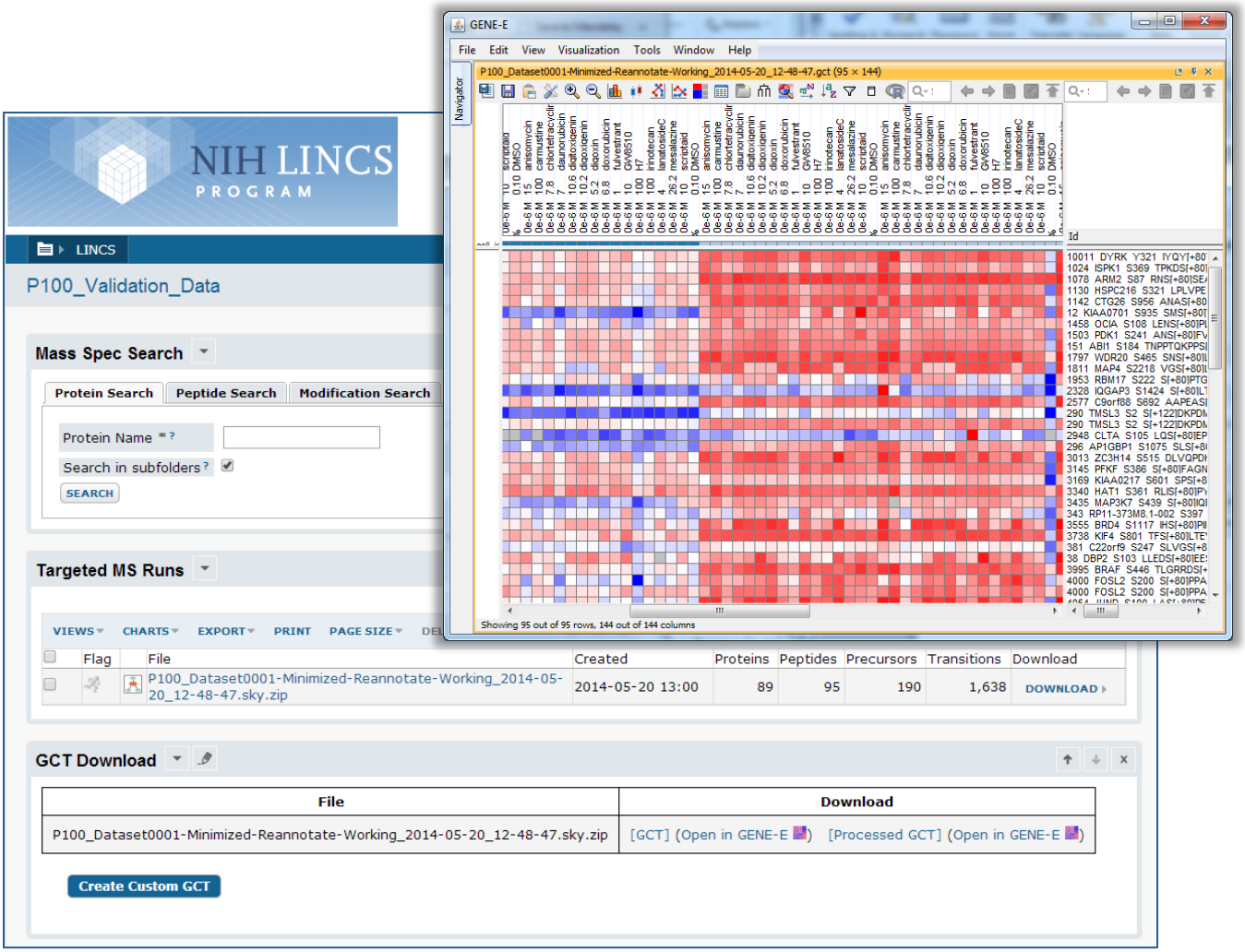
Supplementary Figure 8. Panorama was used to import Excel spreadsheets containing results from participants in the ABRF sPRG study that did not use Skyline for data processing. Results from custom Excel files uploaded to Panorama can also be used as input to analysis scripts executed through the R programming language interface in LabKey Server.



			Sample ID	MSManufacturer	Instrument	Software	Number Of Peptides	Log2Peak Area Ratio Correlation With Consensus Area Ratio	Number Of Heavy Peptides (Retention Time)	Retention Time Correlation With Mean Percentile Rank
	EDIT	DETAILS	az6505	Thermo	QExactive	Skyline	1000	0.61	988	0.91
	EDIT	DETAILS	azh139	AB Sciex	TripleTOF 5600	Skyline	955	0.80	954	1.00
	EDIT	DETAILS	ng1811	Thermo	Orbitrap Velos	Skyline	863	0.85	940	0.99
	EDIT	DETAILS	67gh82			Skyline	839	0.82	948	0.99
	EDIT	DETAILS	47886e	Thermo	Orbitrap Velos	Skyline	836	0.83	922	0.99
	EDIT	DETAILS	260402	AB Sciex	TripleTOF 5600	Unknown	825	0.79	825	0.98
	EDIT	DETAILS	5244gp	AB Sciex	TripleTOF 5600	Skyline	820	0.10	527	0.47
	EDIT	DETAILS	11d75p	Thermo	Orbitrap Velos	Skyline	810	0.78	798	0.96
	EDIT	DETAILS	77777A			Unknown	797	0.84		
	EDIT	DETAILS	1001es	Thermo	Orbitrap Velos	Skyline	797	0.16	239	0.42
	EDIT	DETAILS	12045q	Thermo	QExactive	Skyline	783	0.38	270	0.62
	EDIT	DETAILS	lua330	AB Sciex	QSTAR XL	Skyline	772	0.15	562	0.26
	EDIT	DETAILS	131003	Thermo	QExactive	Skyline	770	0.28	600	0.52
	EDIT	DETAILS	AS6842-01	Thermo	Orbitrap Elite	MaxQuant	764	0.83		
	EDIT	DETAILS	356180	Thermo	QExactive	Skyline	762	0.87	820	0.99
	EDIT	DETAILS	1e356x	Thermo	Orbitrap Elite	Skyline	756	0.85	932	0.99
	EDIT	DETAILS	ms1182	Thermo	Orbitrap XL	Skyline	729	0.27	565	0.81
	EDIT	DETAILS	MFIG13	Thermo	Orbitrap Velos	MaxQuant	718	0.78	718	0.98
	EDIT	DETAILS	sPRG02	Thermo	QExactive	Skyline	705	0.81	685	0.91
	EDIT	DETAILS	12358f	Bruker	Impact	Skyline	668	0.79	786	1.00
	EDIT	DETAILS	sPRG01	AB Sciex	TripleTOF 5600	Skyline	659	0.74	621	0.98
	EDIT	DETAILS	pl1377	Thermo	Orbitrap Velos	Skyline	650	0.88	718	0.99
	EDIT	DETAILS	02940a	Thermo	LTQ-FT	Skyline	642	0.51	707	0.71

Supplementary Figure 9. A custom summary results table, uploaded to the ABRF sPRG project, enables rapid access to individual participant results as well as an overview of the project. Custom formatting, such as conditional color coding of cells as seen in this figure, can be applied to Excel files uploaded to LabKey Server.

Supplementary Figure 10. Examples of data quality assessment plots generated for the ABRF sPRG project using the R programming language interface available in Panorama.



Supplementary Figure 11. Scripts developed in the R programming language were written to generate Gene Cluster Text (GCT) files from annotated Skyline documents uploaded to the LINCS project on PanoramaWeb. A custom interface was designed, using tools available in LabKey Server, to provide easy access to the generated GCT files, as well as provide the option to create custom GCT files by filtering on available annotations. GCT files can be visualized and explored in the GENE-E viewer (http://www.broadinstitute.org/cancer/software/GENE-E/).

Supplementary Note 1: An introductory tutorial covering a broad range of features in Panorama

Panorama Sharing Skyline Documents

Panorama is a freely available, open-source web server database application for targeted proteomics assays that integrates into a Skyline proteomics workflow. It has been implemented as a module within LabKey Server, an open-source bioinformatics data management platform with extensive support for proteomics data and a security model rich enough to support clinical studies.

Panorama can be installed by laboratories and organizations on their own servers. You can visit the LabKey Server installation documentation (https://www.labkey.org/wiki/home/Documentation/page.view?name=installServerDemo) for the available installation options. For this tutorial you will use the Panorama server hosted at the University of Washington (panoramaweb.org) where users can request free projects and have full administrative rights to configure data organization and security.

This tutorial is an introduction to Panorama and covers the following areas:

- Requesting a project on panoramaweb.org
- Data organization and folder management in Panorama
- Publishing Skyline documents to Panorama
- Data display options and searching results uploaded to Panorama
- Providing access to collaborators and other groups

Getting started

To start this tutorial, download the following ZIP file:

https://panoramaweb.org/tutorials/PanoramaSharing.zip

Extract the files in it to a folder on your computer, like:

C:\Users\bschilling\Documents

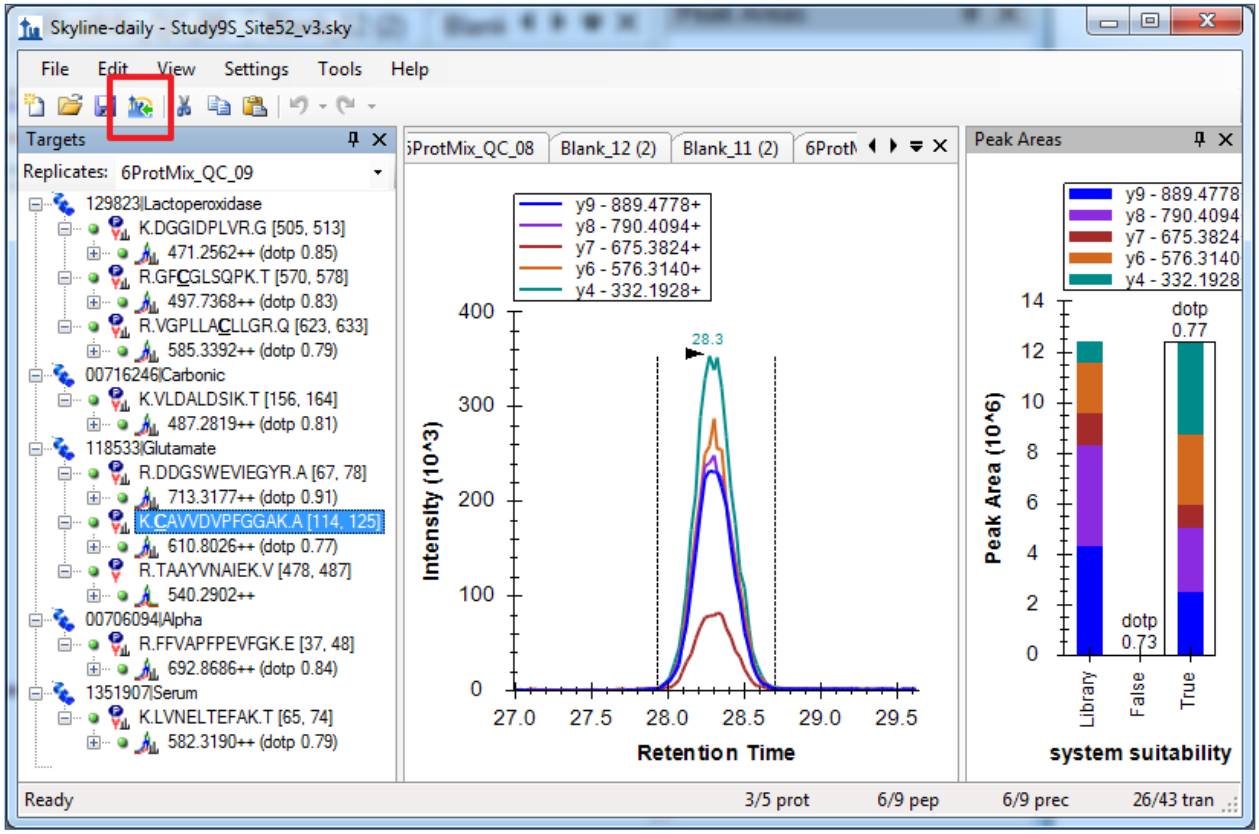
This will create a new folder:

C:\Users\bschilling\Documents\PanoramaSharing

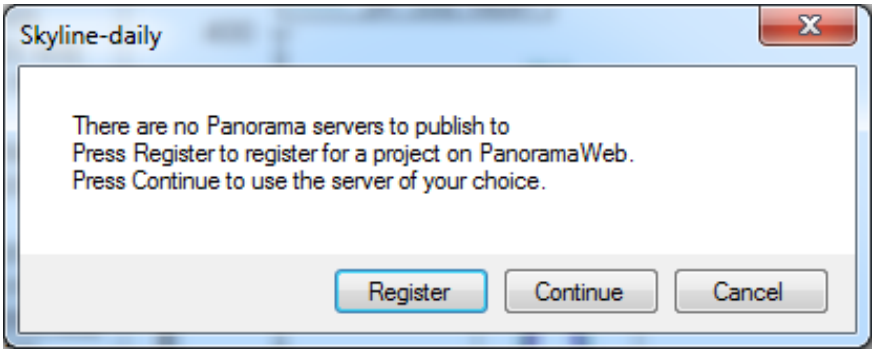
It will contain all the files necessary for this tutorial.

Requesting a project on panoramaweb.org

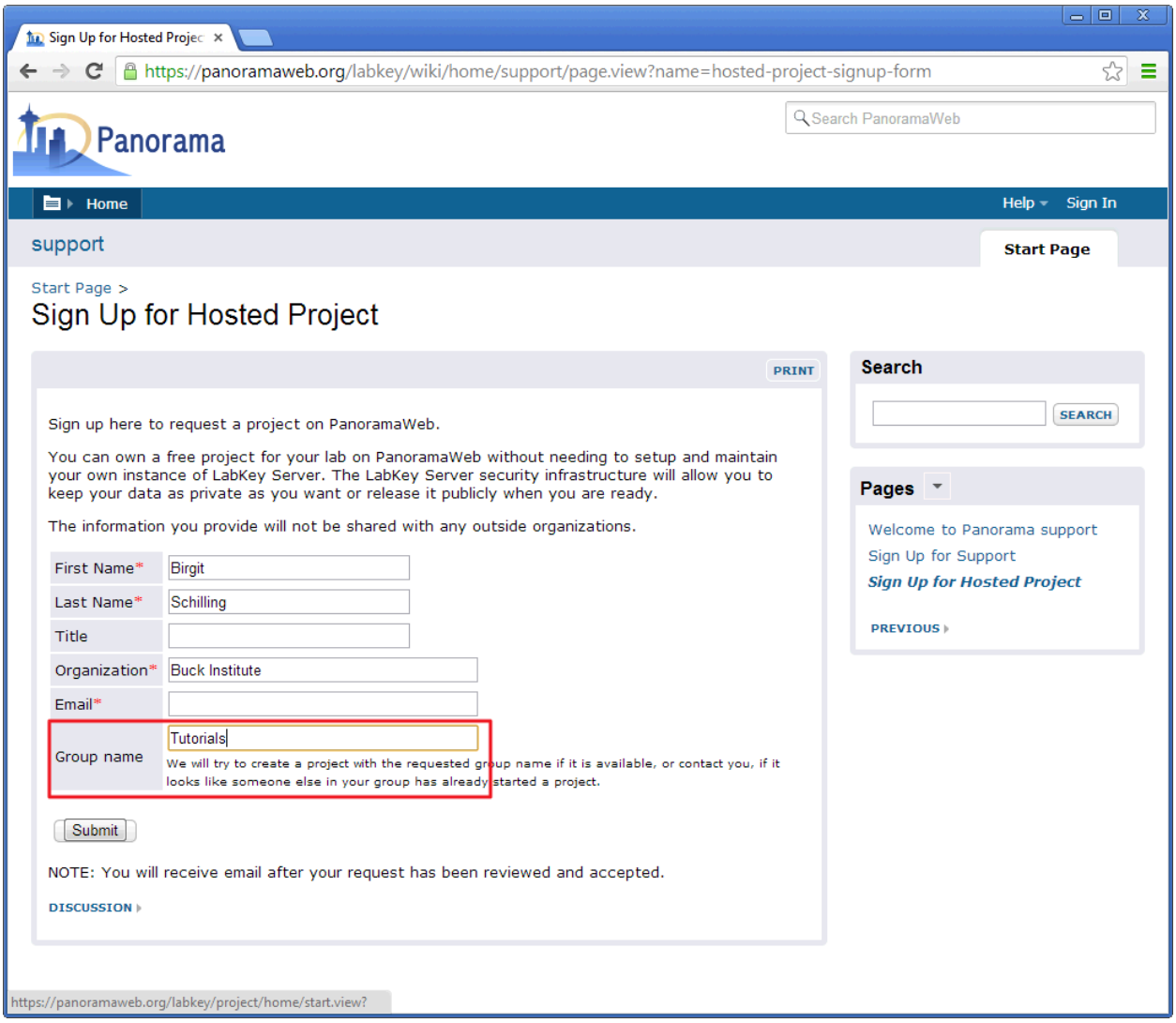
Open the Study9S_Site52_v3.sky file in Skyline either by double-clicking on the file in Windows Explorer or by going through the **File > Open** menu in Skyline. This file contains results that are part of a recent CPTAC multi-site SRM-MS system suitability study (Abbatiello et al., *Mol. Cell. Proteomics* 2013). The data in this document was collected at Site 52. To publish this document to Panorama, click on the Publish to Panorama button  in the toolbar, as shown below. Alternatively, on the **File** menu, click **Publish to Panorama**.



Since you have not yet registered a Panorama server in Skyline, you will see the following message:



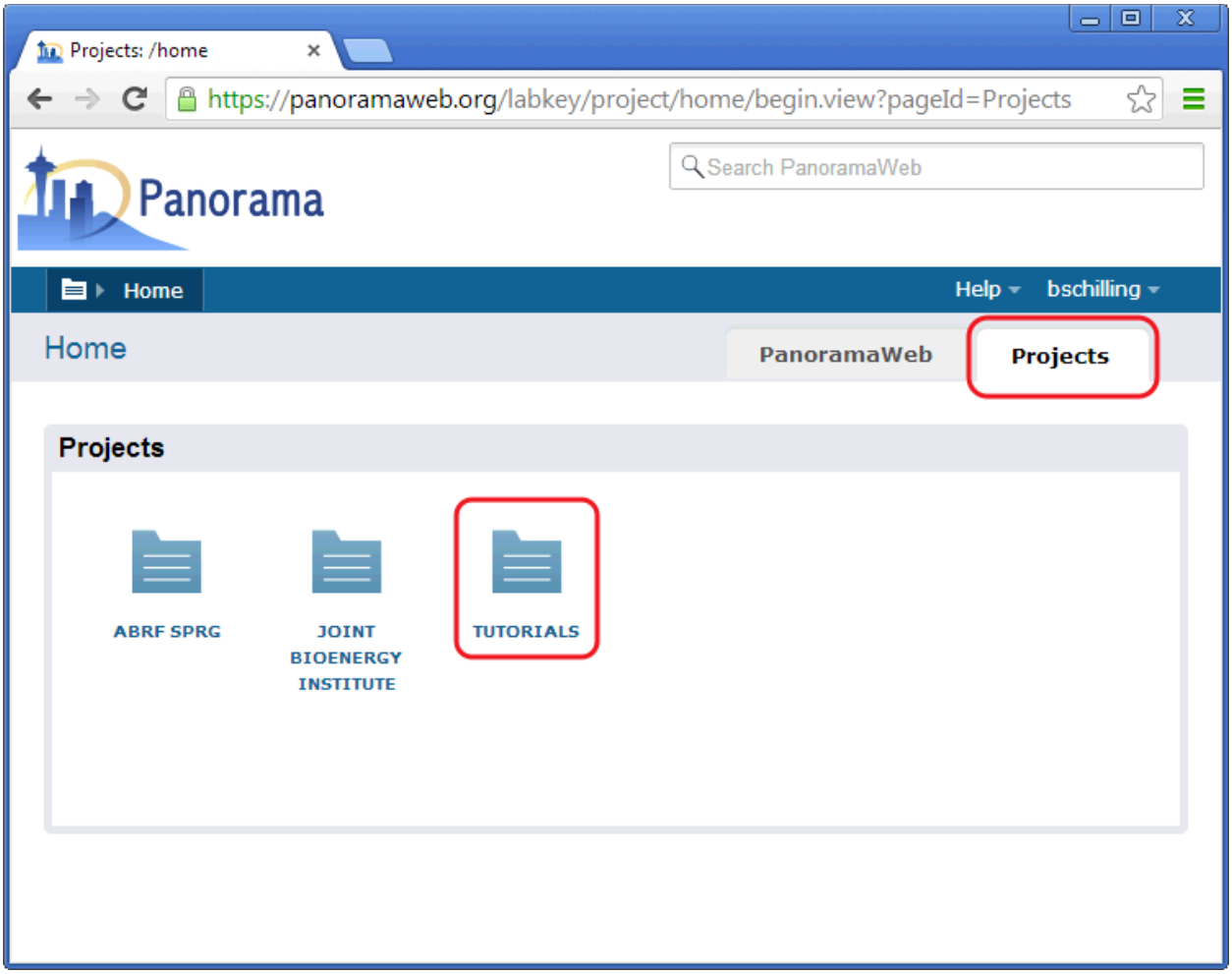
If you have an existing account on a Panorama server, and you would like to use that, click on **Continue** and enter the server details in the **Edit Server** form Skyline presents. If you do not have an existing Panorama account, click on **Register** to request a project on the PanoramaWeb server hosted at the University of Washington. This will open the project sign-up page on panoramaweb.org in a web-browser as shown below.



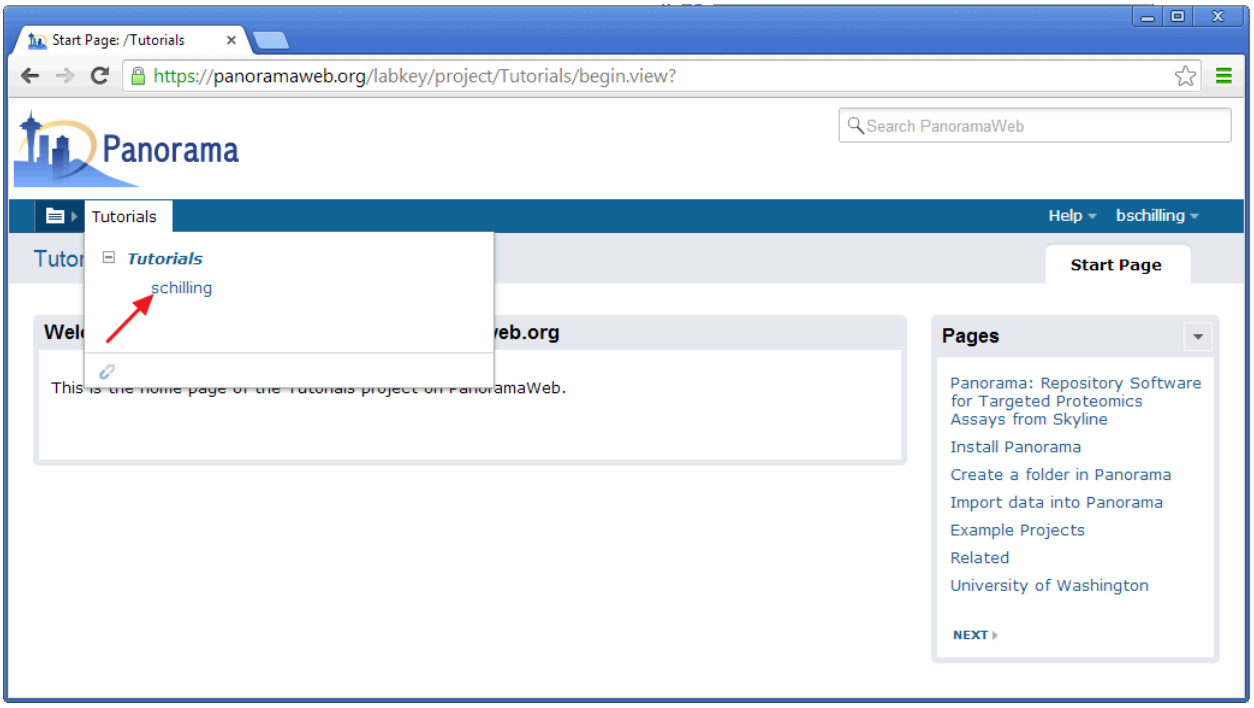
- Fill out the form and be sure to enter a **Group name**.
- Click **Submit**.

The group name is used to create projects on panoramaweb.org. The Panorama team reviews all project requests and, upon approval, creates a project with the requested group name. If you are looking for a space to work through this tutorial and explore Panorama, please enter 'Tutorials' in the **Group name,** and a folder with full administrative rights will be created for you in the 'Tutorials' project on panoramaweb.org. Otherwise, enter your preferred project name. Once your project or folder has

been created, you will receive a welcoming email with a link to setup a password for your account. After you have completed the registration process you can sign-in to panoramaweb.org. On the home page, click the **Projects** tab to see a list of all the projects to which you have access on panoramaweb.org. If you entered 'Tutorials' in the project sign-up form, you will see the 'Tutorials' project where a folder was just created for you.



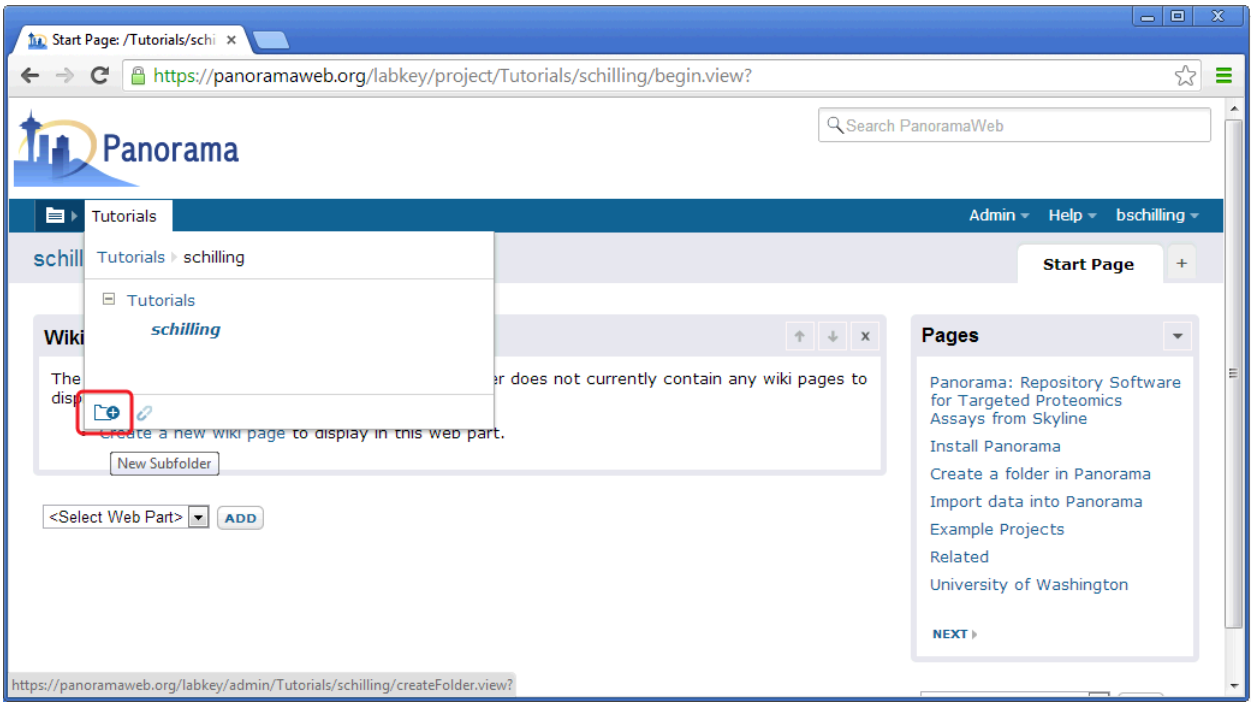
Click on the 'Tutorials' project to go to the project homepage. On the project homepage, hover over the project name in the menu bar below the Panorama icon and click on the folder that was created for you (e.g. 'schilling'). You will have full administrative rights in your folder, so you will be able to create a folder structure for organizing your Skyline documents, and configure user access to the sub- folders.



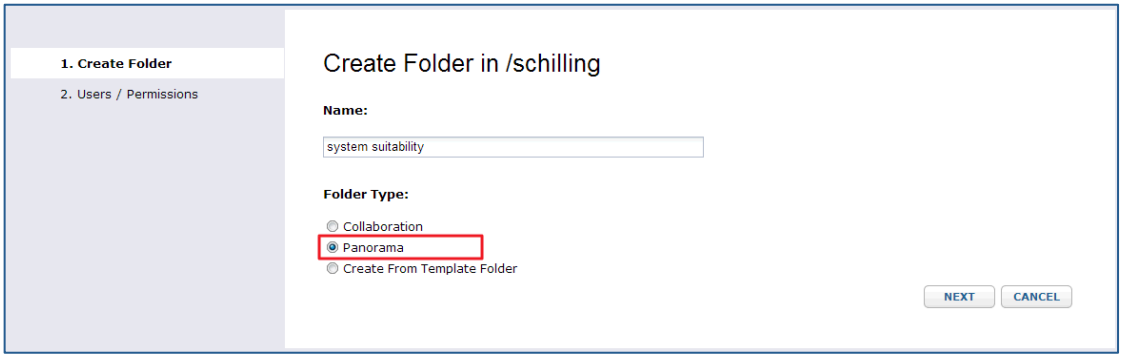
Creating a subfolder in Panorama

Skyline documents cannot be uploaded to the root project folder on panoramaweb.org or your folder in the 'Tutorials' project. So, you will create a sub-folder for uploading the Skyline document that you opened earlier. As a project administrator, you would typically organize your project workspace to have subfolders for each project and/or each researcher in your laboratory or organization. Follow these steps to create a 'system suitability' sub-folder in your folder in the "Tutorials" project:

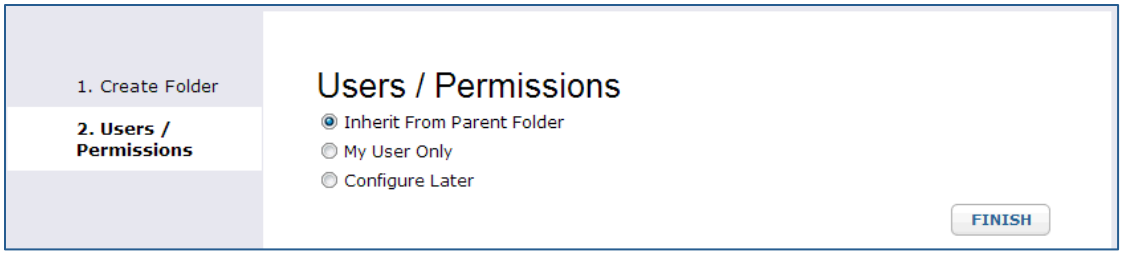
- Navigate to your folder home page, if you are not already there.
- Hover over the project name in the menu bar below the Panorama icon and click on the new folder icon shown in the image below.



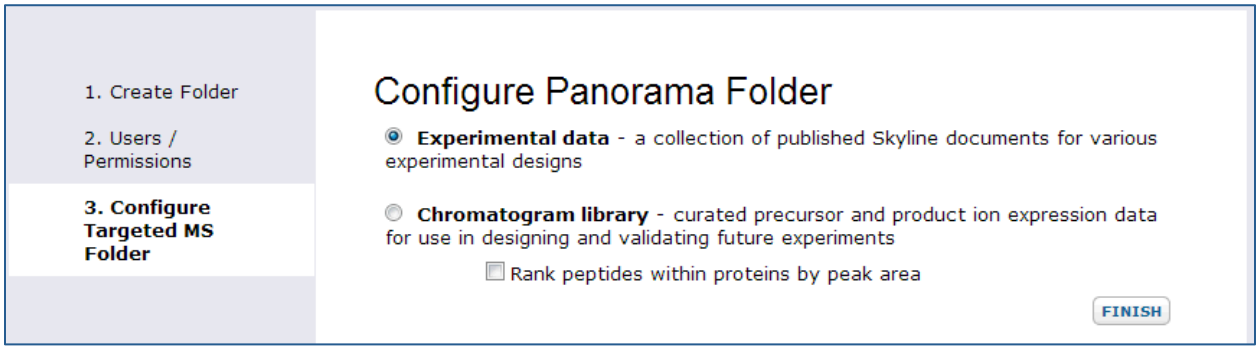
- In the **Create Folder** form, enter 'system suitability' in the folder **Name** field.
- Select the **Panorama** option under **Folder Type**. This is the folder type that should be selected for all workflows supported by Skyline (SRM-MS, MS1 filtering or MS2 based projects).
- Click the **Next** button.



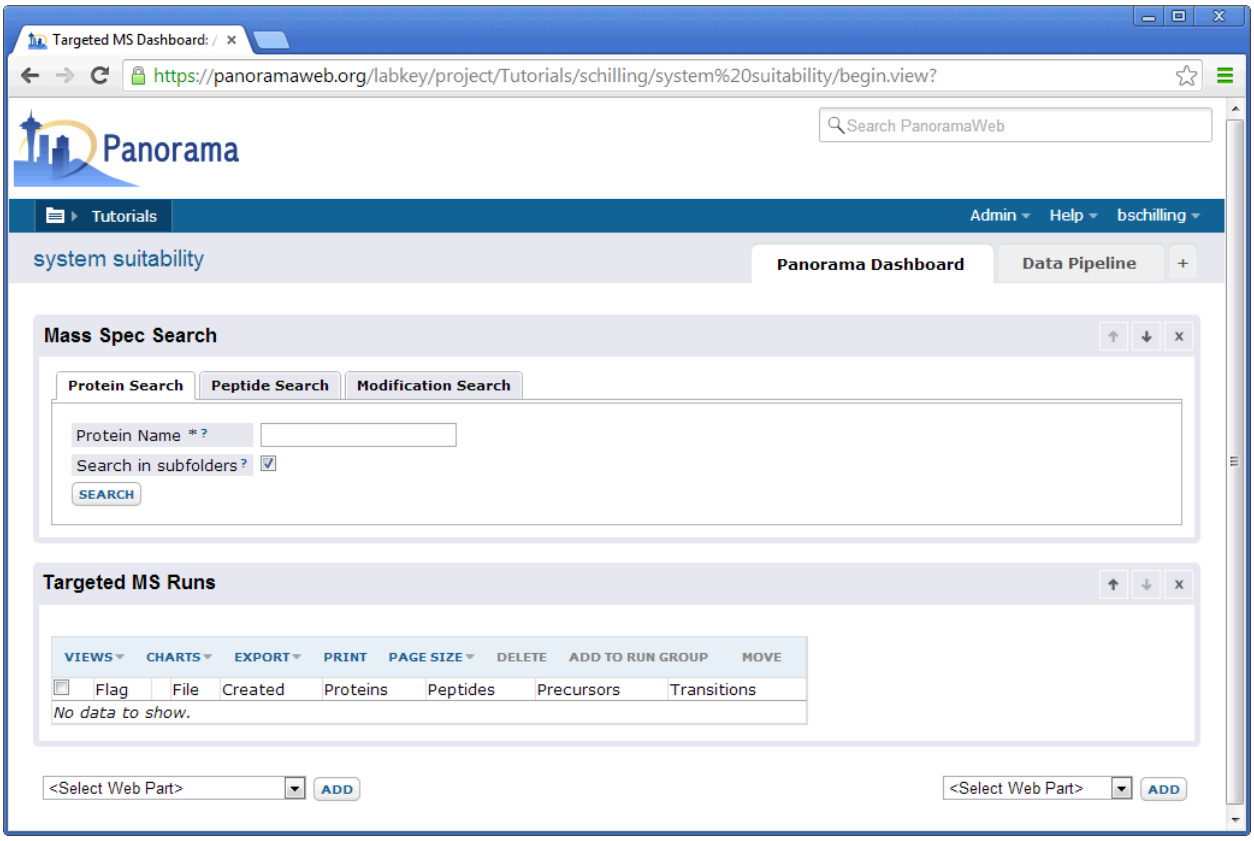
- On the **Users / Permissions** page accept the default selection and click **Next**. You will configure user permissions on this folder later.



- The next page (**Configure Panorama Folder**) asks you to choose the type of Targeted MS folder you would like to create. Panorama offers two choices here. For this tutorial accept the default **Experimental data** option. This option is meant for folders that serve as a repository of Skyline documents, useful for collaborating, sharing and searching across multiple experiments.
- Click the **Finish** button.



You will be taken to the home page of the 'system suitability' sub-folder that should look as follows:

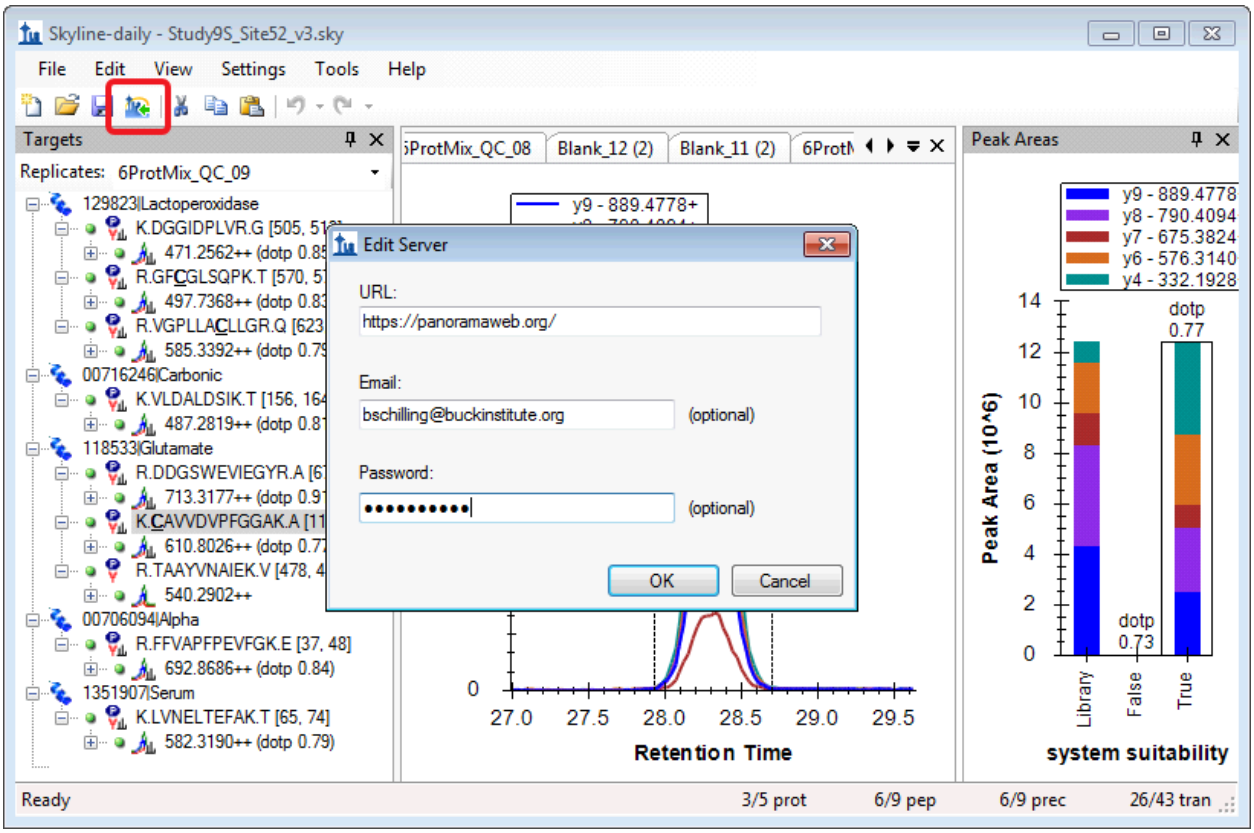


You are now ready to upload Skyline documents to this folder.

Publishing data from Skyline to Panorama

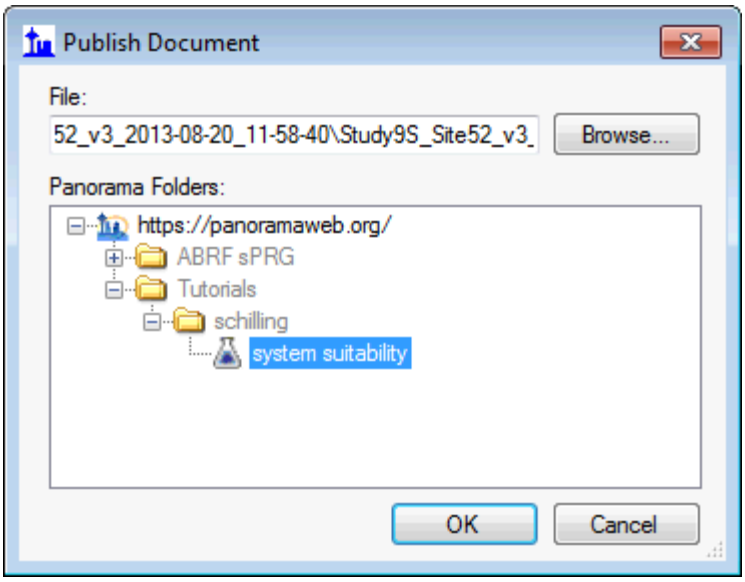
To publish your first Skyline document to PanoramaWeb, perform the following steps:

- Open Study9S_Site52_v3.sky in Skyline if it is not already open.
- If the **Edit Server** form is not already open, open it by clicking on the  Publish to Panorama button and clicking **Continue** in the resulting form.
- In the **Edit Server** form, enter the email address and password with which you registered on panoramaweb.org. The server URL should already be entered as https://panoramaweb.org.
- Click the **OK** button.



Skyline will now display a form with the folder hierarchy on panoramaweb.org. Only the folders to which you have access will be displayed.

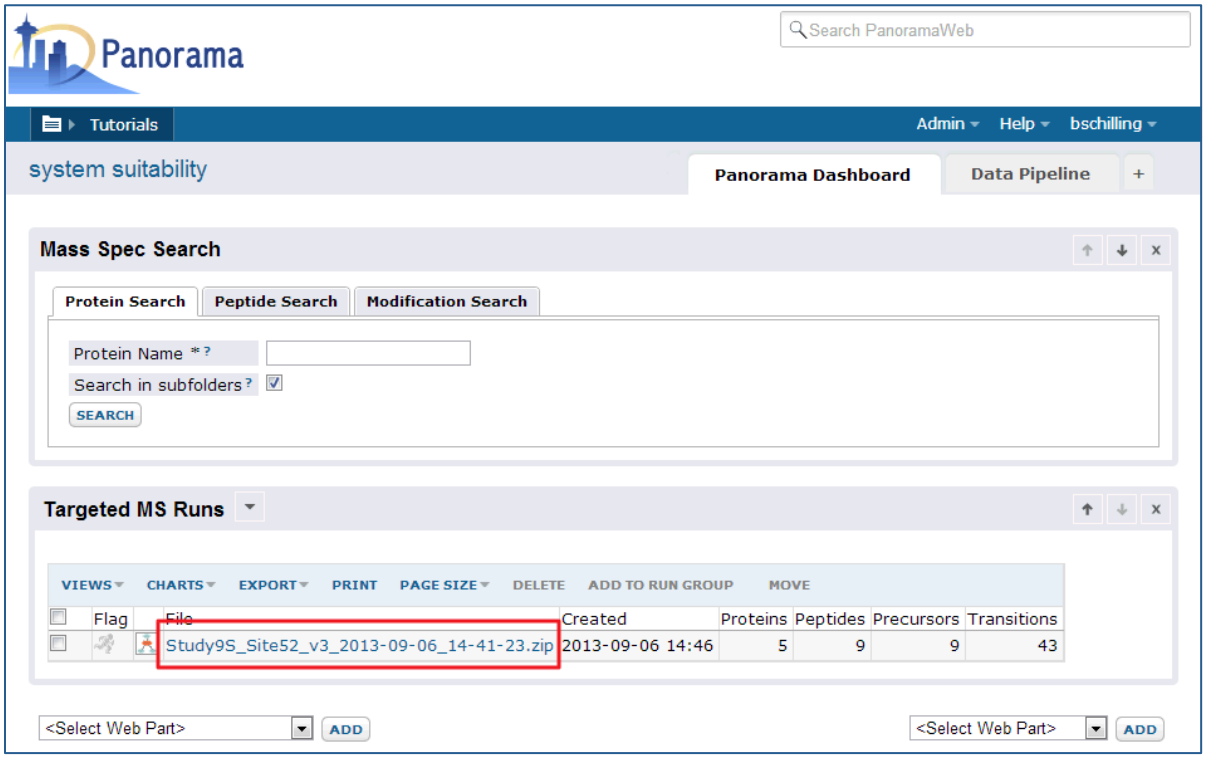
- Navigate to and select the 'system suitability' folder.
- Click the **OK** button.



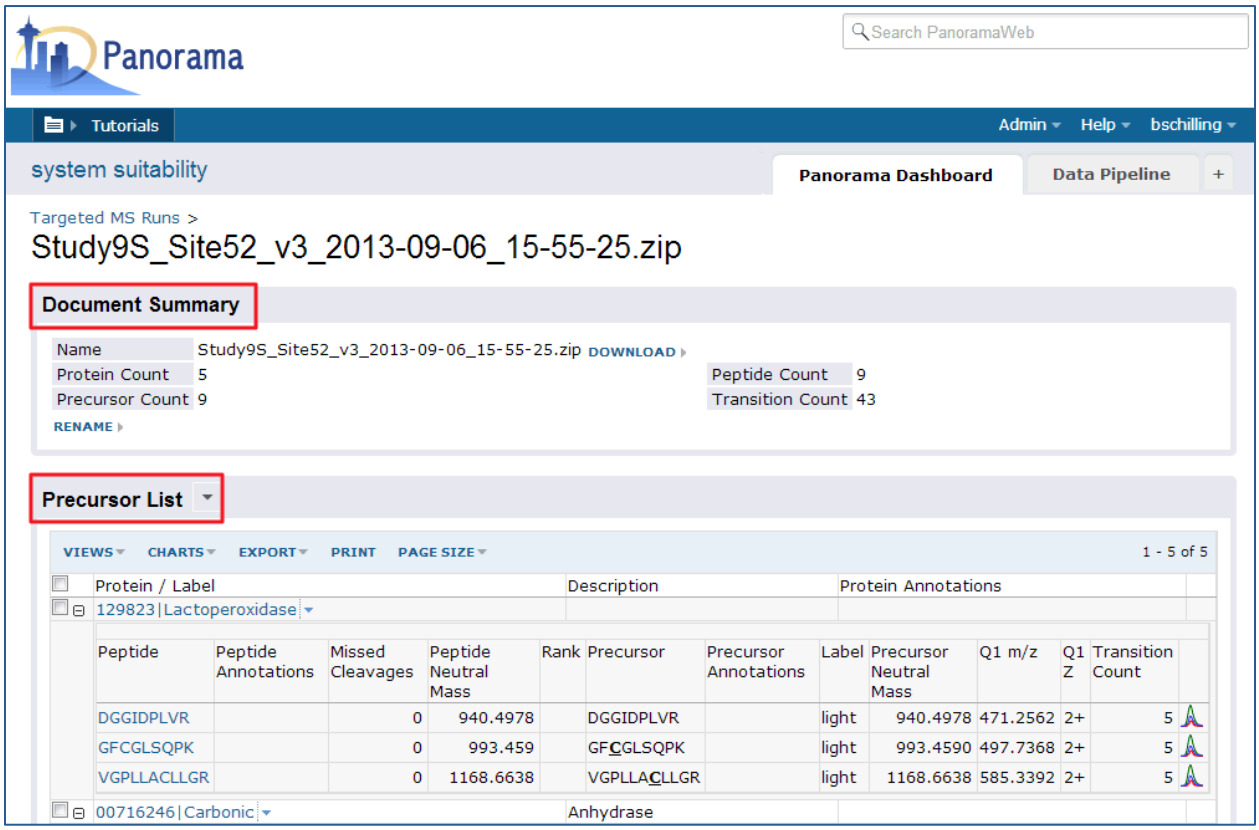
Skyline will create a ZIP archive of the files for your document and upload the ZIP file to panoramaweb.org, where it will be imported into the Panorama database.

Viewing data in Panorama

Once your document has been imported into Panorama, go back to the web-browser where the home page of the 'system suitability' folder should still be open. Refresh the page (F5 in most browsers) and you will see the file that you just uploaded, in the **Targeted MS Runs** box.



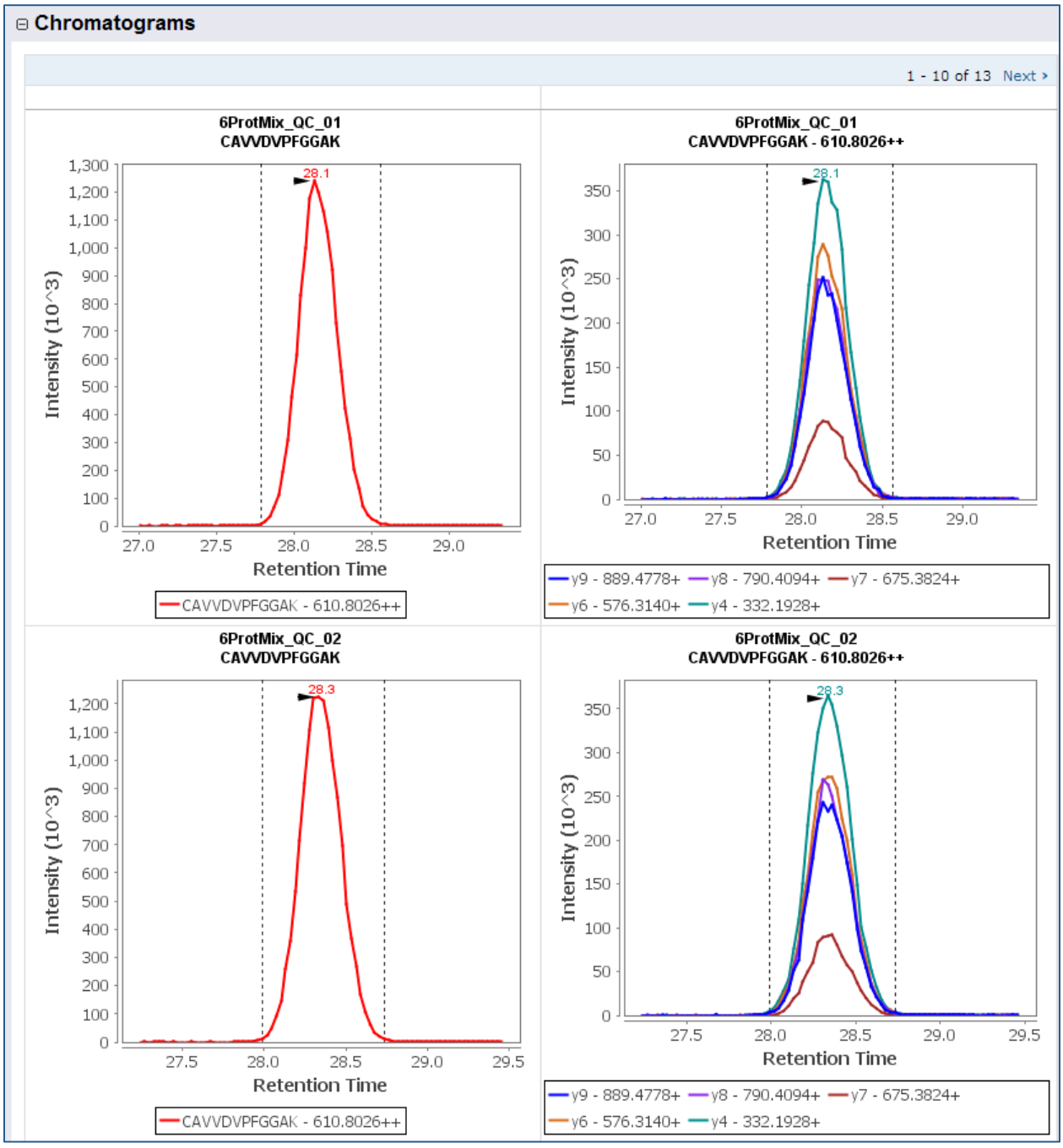
Click on the file name to view the document details. The **Document Summary** box shown in image below gives you a summary of the number of proteins, peptides and transitions in your document.



Below the **Document Summary** box you can view a list of all the proteins, peptides and precursors in your document, along with some of the information imported from the Skyline document, such as a modified peptide sequence, m/z and charge. You can click on a protein or peptide to view more details, including chromatograms and some of the other graphs that are also available in Skyline. Scroll down on the page and click on the peptide CAVVDVPFGGAK. This will take you to a page that has the following details for this peptide:

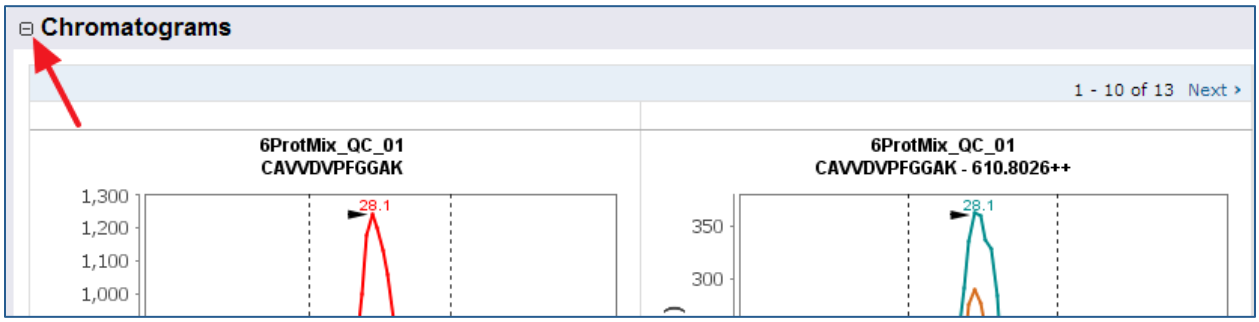
1. Chromatograms to show quantitative data quality

This peptide was measured in 10 QC system suitability replicates and there were 3 blank acquisitions. On this page you can view the chromatograms for this peptide in all the replicates. The image below shows the precursor and transition chromatograms in the replicates QC_01 and QC_02. There is one row of chromatograms for each replicate in your Skyline document, where the first column has the total precursor chromatogram and the subsequent columns have transition chromatograms. If this precursor was measured in a light as well as a heavy-labeled from, transition chromatograms for both the forms would be displayed side-by-side.

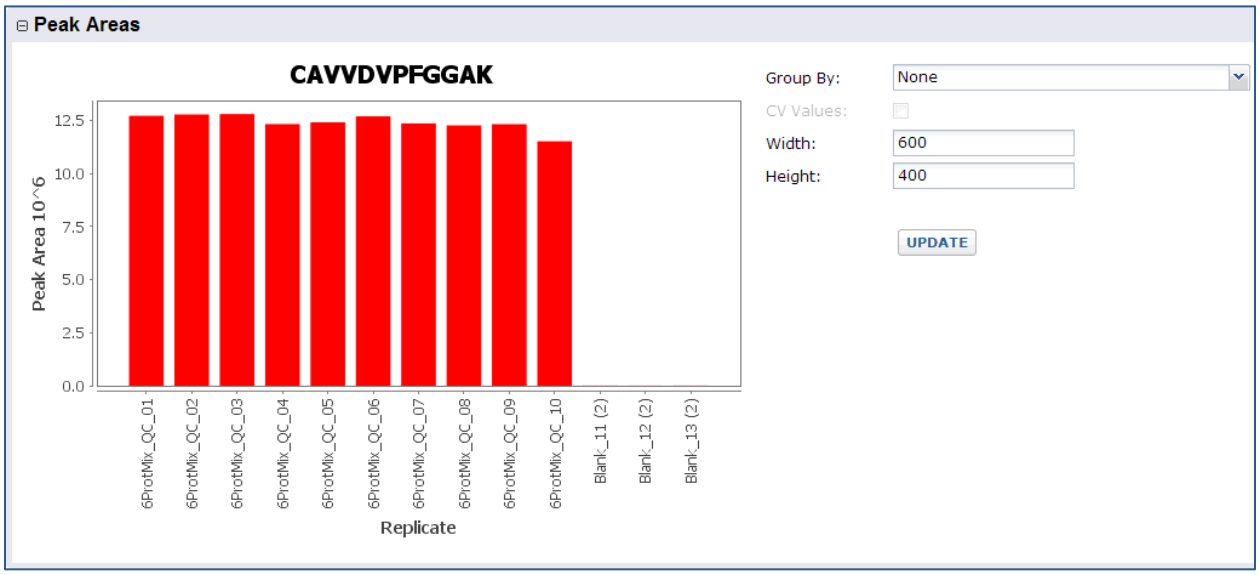


2. Peak area replicate graph

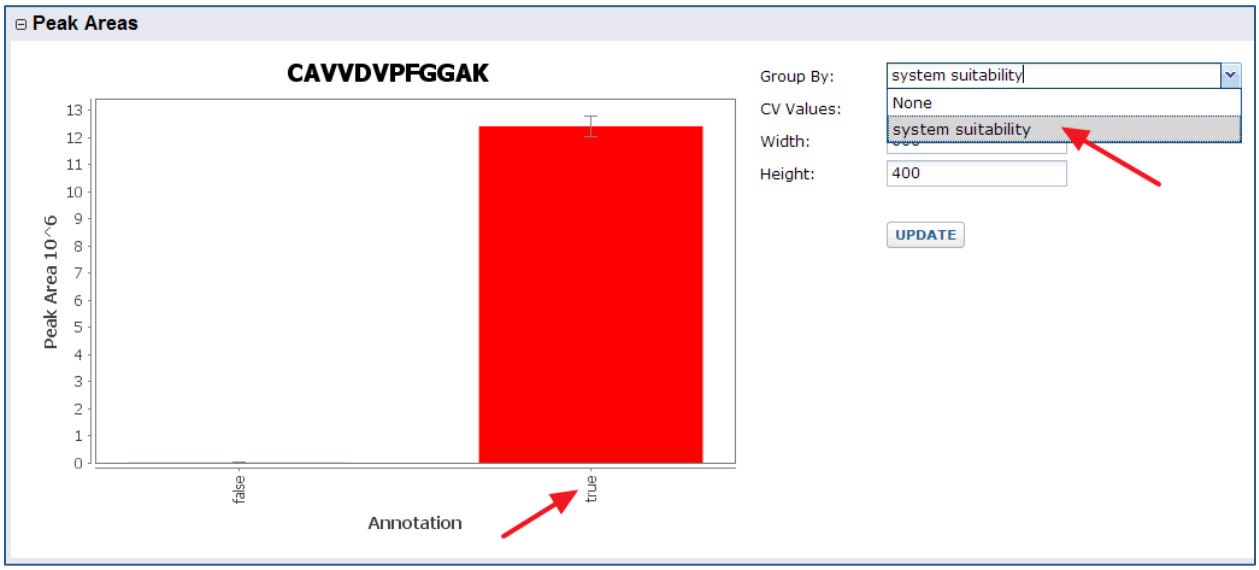
Scroll further down on the page or collapse the Chromatograms box by clicking on the icon at the top left corner of the box as shown in the image.



You will now see a graph displaying the peak area for this peptide in the 13 replicates. This graph is similar to what you would see in Skyline for this peptide.



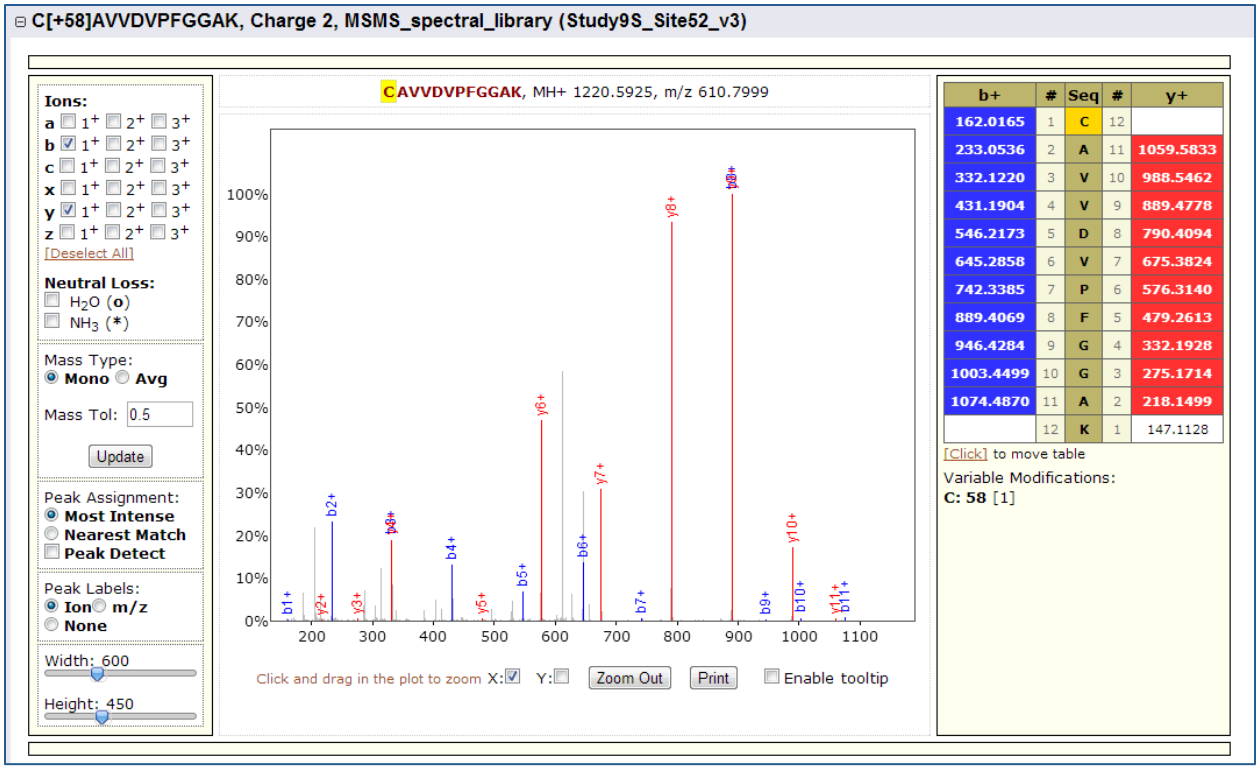
As in Skyline, Panorama also allows grouping peak areas by custom replicate annotations. In this Skyline document researchers used a custom 'system suitability' annotation that was set to 'true' for the system suitability replicates and 'false' for the blank acquisitions. You can group the peak areas by selecting 'system suitability' from the pull-down combo-box next to **Group By**. Click **Update** to see peak areas grouped by the 'system suitability' annotation.



In the graph above, the red bar labeled 'true' represents the 10 system suitability runs, and the small error bar indicates that there was very little variability in the measurements for this peptide in the 10 replicates. You can also switch to a Coefficient of Variation (CV) view by checking the **CV Values** box and clicking on **Update** again.

3. Library MS/MS spectrum viewer

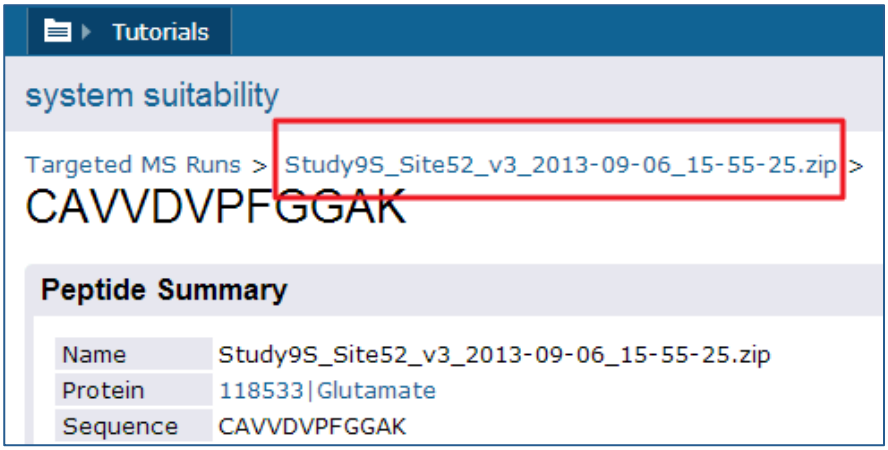
Scroll down further on the peptide details page to see an interactive spectrum viewer displaying the MS/MS spectrum for **CAVVDVPFGGAK** from the spectrum library associated with this Skyline document. The name of the library is displayed in the title bar of the viewer. The viewer allows you to zoom in by clicking and dragging your mouse over the spectrum. You can also customize the view by selecting the ion types and charge states you want to display from the options menu to the left of the spectrum. A color-coded table to the right of the spectrum lists all the fragment ion m/z values for the peptide, with colored boxed representing the matching fragment ions found in the library spectrum. These spectral library views can be used to support publications in peer-reviewed journals or share data with collaborators.



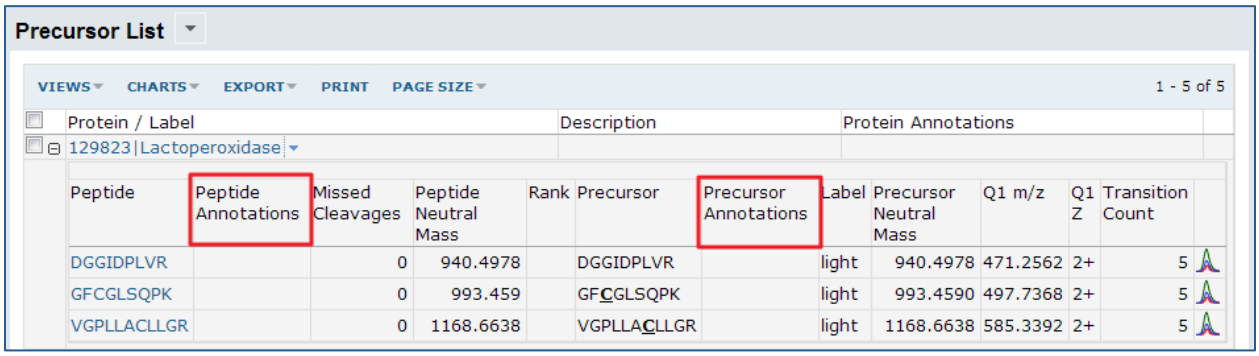
b+	#	Seq	#	y+
162.0165	1	C	12	
233.0536	2	A	11	1059.5833
332.1220	3	V	10	988.5462
431.1904	4	V	9	889.4778
546.2173	5	D	8	790.4094
645.2858	6	V	7	675.3824
742.3385	7	P	6	576.3140
889.4069	8	F	5	479.2613
946.4284	9	G	4	332.1928
1003.4499	10	G	3	275.1714
1074.4870	11	A	2	218.1499
	12	K	1	147.1128

Customizing views in Panorama

On your web-browser click the back button, or scroll to the top of the page and click on the ZIP file name in the navigation trail just under your folder name as shown in the image below. This will take you back to the documents details page.

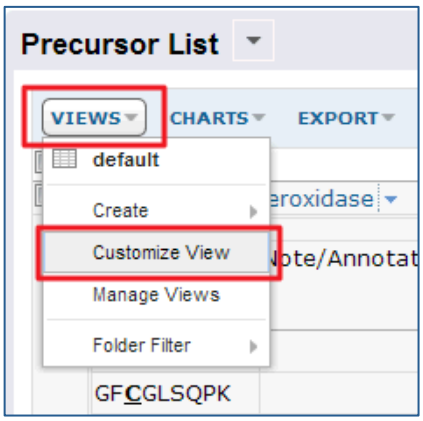


LabKey Server, on top of which Panorama is built, allows customizations of all tabular views. You can select which columns you would like to see, order them according to your preference, and define a sorting order. The **Precursor List** table on this page has blank annotation columns since the researchers did not define any custom peptide or precursor annotations in this Skyline document.



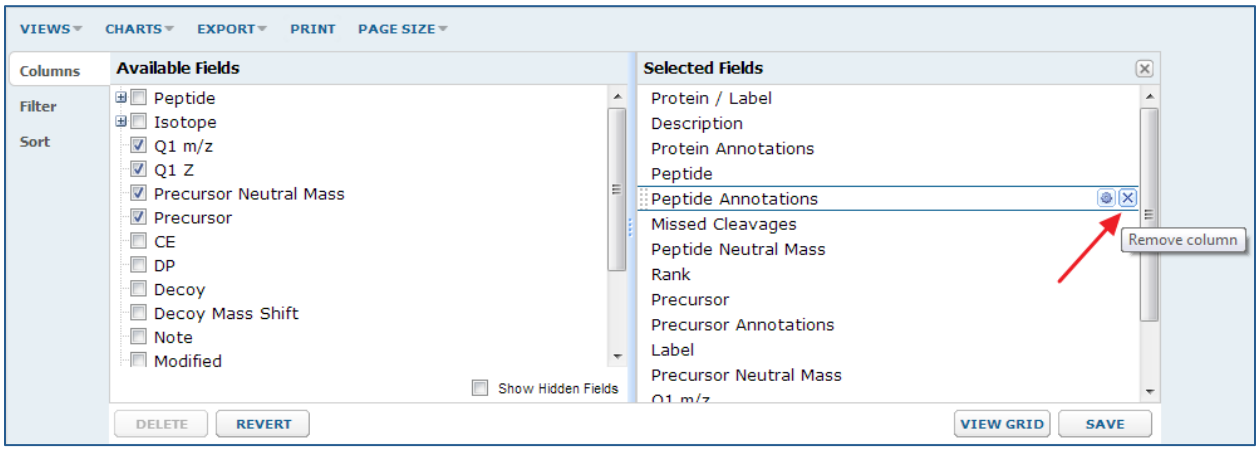
To hide these columns from the default view preform the following steps:

- Click on the **Views** button at the top of the table, just under the **Precursor List** heading.
- Select **Customize View** from the drop-down menu.



In the customization form that appears, you will see a **Selected Fields** column that shows the fields that are currently displayed.

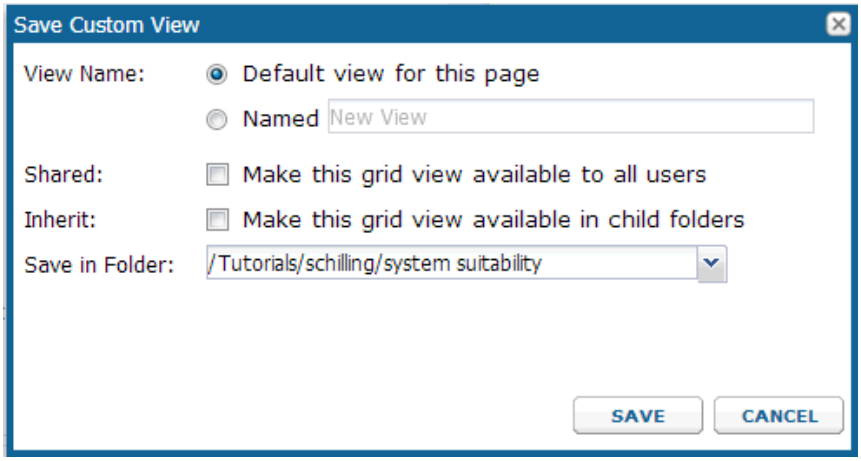
- Hover over **Peptide Annotations** below **Peptide** in the **Selected Fields** and click on the remove icon .
- Do the same for **Precursor Annotations** below **Precursor**.
- Click the **Save** button.



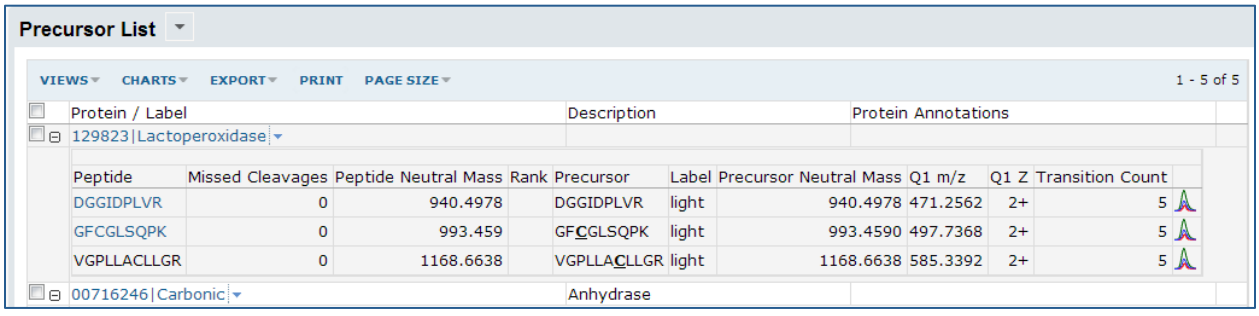
- In the **Save Custom View** form, check **Make this grid view available to all users**.

This will cause all users with access to this folder see the same table columns.

- Click the **Save** button.



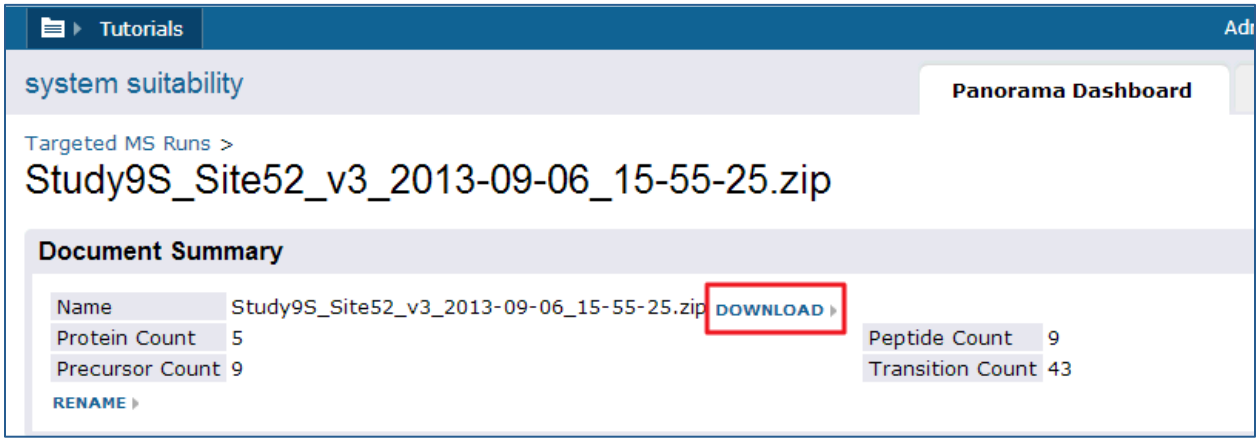
The annotation columns should no longer be displayed, as shown below:



You can view the LabKey Server documentation on customizing views (https://www.labkey.org/wiki/home/Documentation/page.view?name=customViews) for more information.

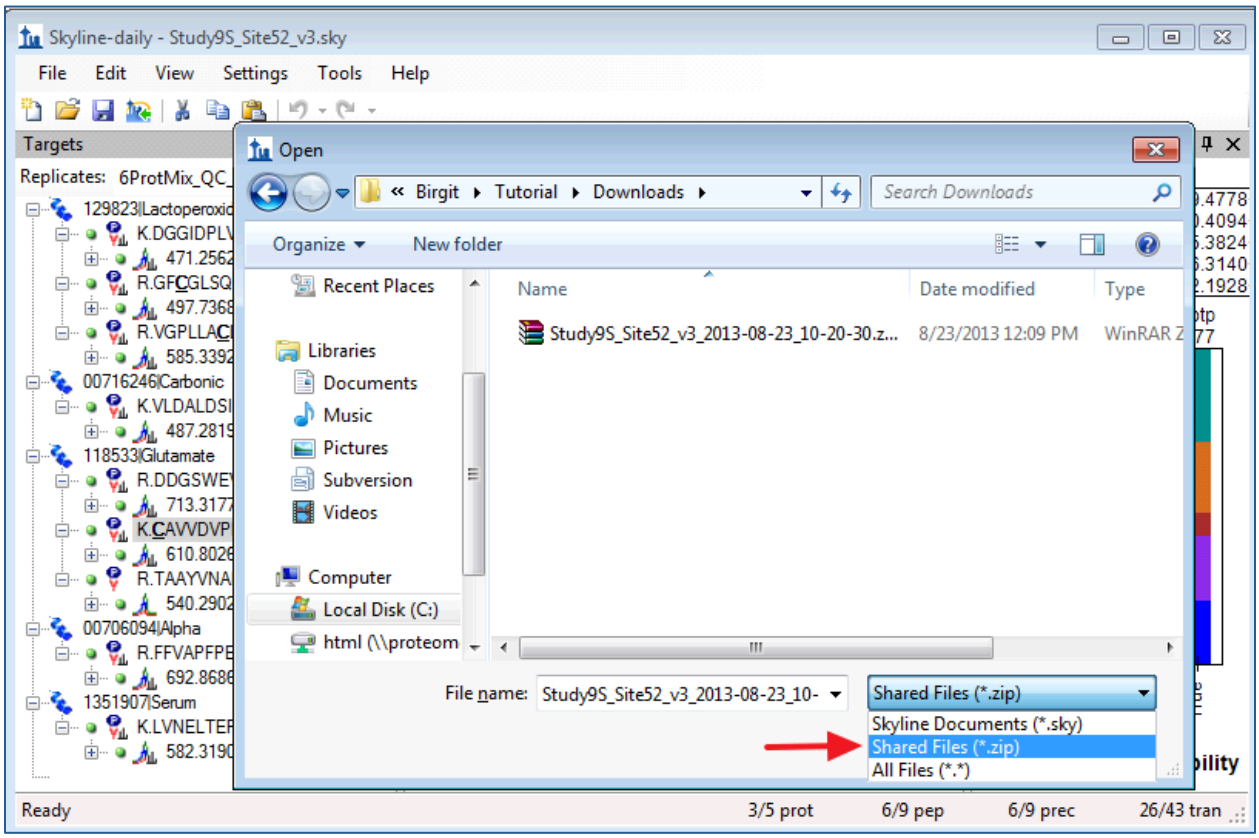
Downloading Skyline files from Panorama

Scroll up, if you need to, to view the **Document Summary** box. You can download the original Skyline ZIP archive that was published to Panorama by clicking on the **Download** link next to the file name.



To open this zip file in Skyline do the following:

- On the **File** menu, click **Open** (Ctrl-O).
- Select **Shared Files (*.zip)** in the **Open** form as shown in the image below



- Navigate to and select the zip file that you downloaded.
- Click the **Open** button.

Publishing to Panorama provides a convenient way to share Skyline documents with other researchers. You will see later in this tutorial how to give other users access to the folders you create in Panorama.

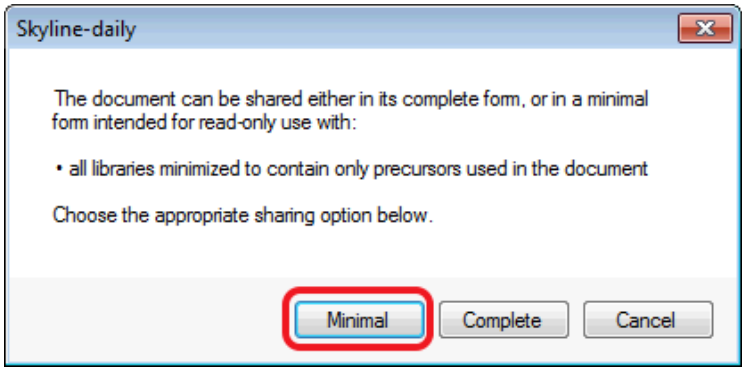
Uploading documents using the Panorama web interface

Using the Skyline **Publish to Panorama** toolbar button or menu item is the most convenient way to get your documents into Panorama. But this can also be done by using the Panorama web interface. Next, you will use the web-interface to upload another document to the 'system suitability' folder.

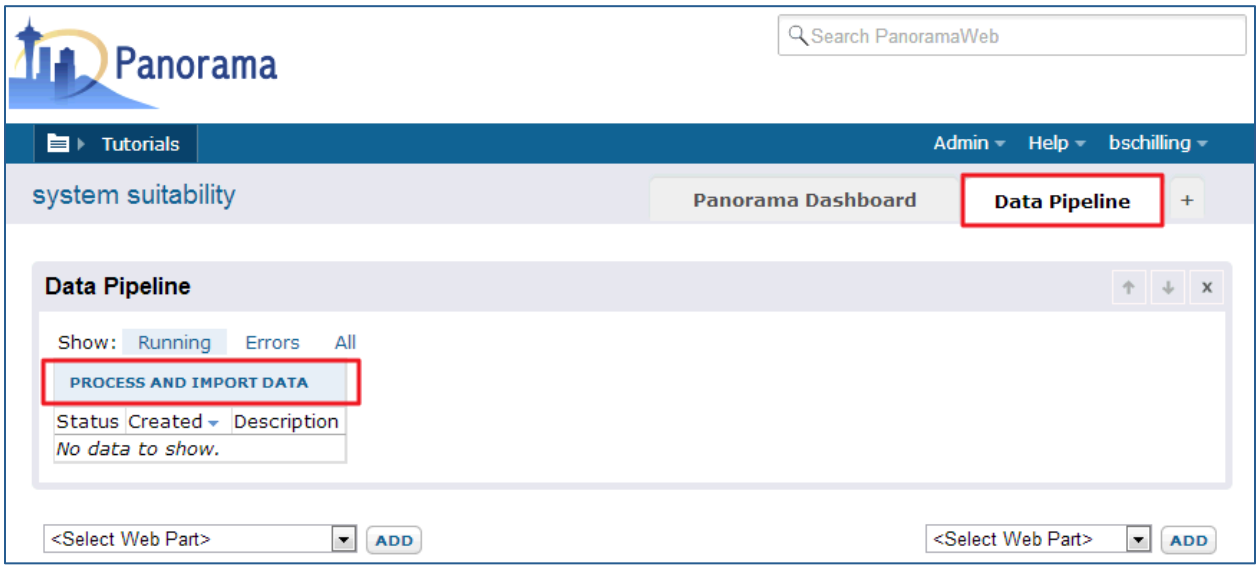
- In Skyline, open the file Study9S_Site54_v2.sky.

This file contains results acquired at another site in the CPTAC system suitability study. Prepare this file for upload to Panorama by doing the following:

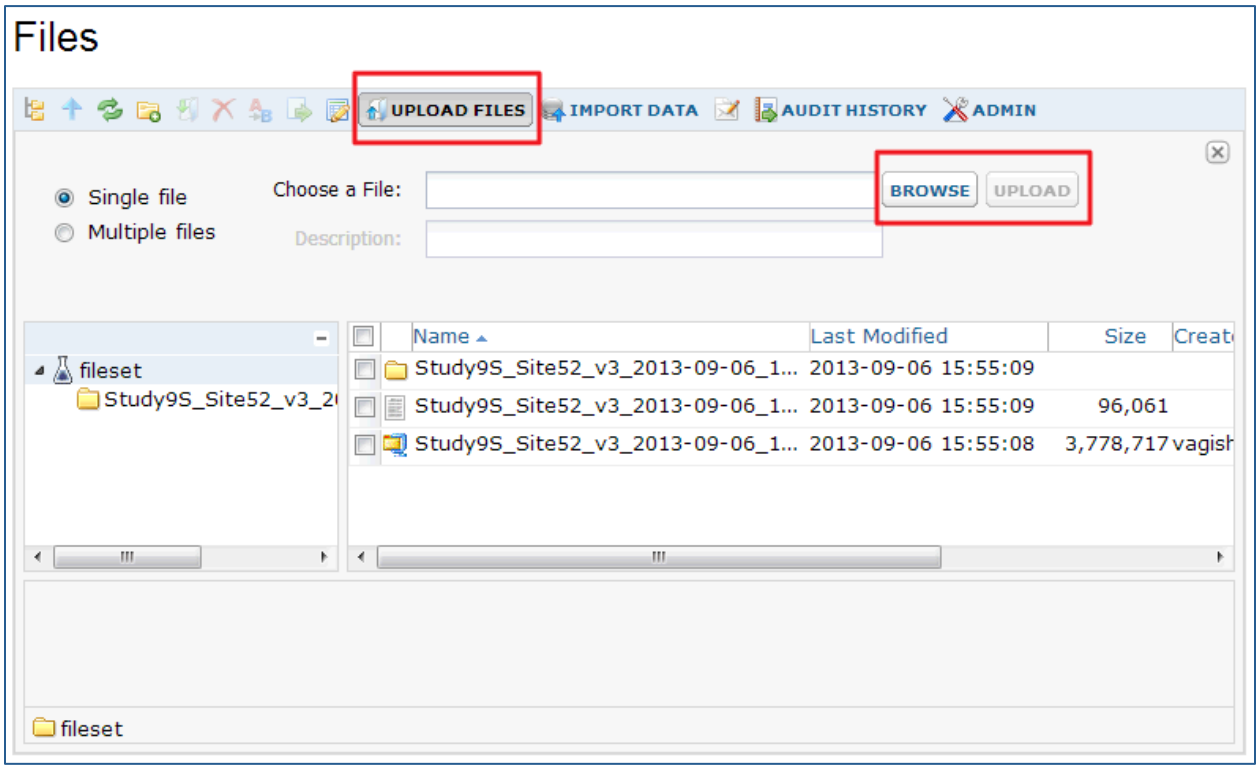
- In the **File** menu, click **Share**.
- Click the **Minimal** button in the form that appears.



- Save the ZIP file on your computer using the **Share Document** form presented by Skyline.
- In Panorama, go to the 'system suitability' folder.
- Click the **Data Pipeline** tab in the upper right corner.
- Click on **Process and Import Data** as shown below.

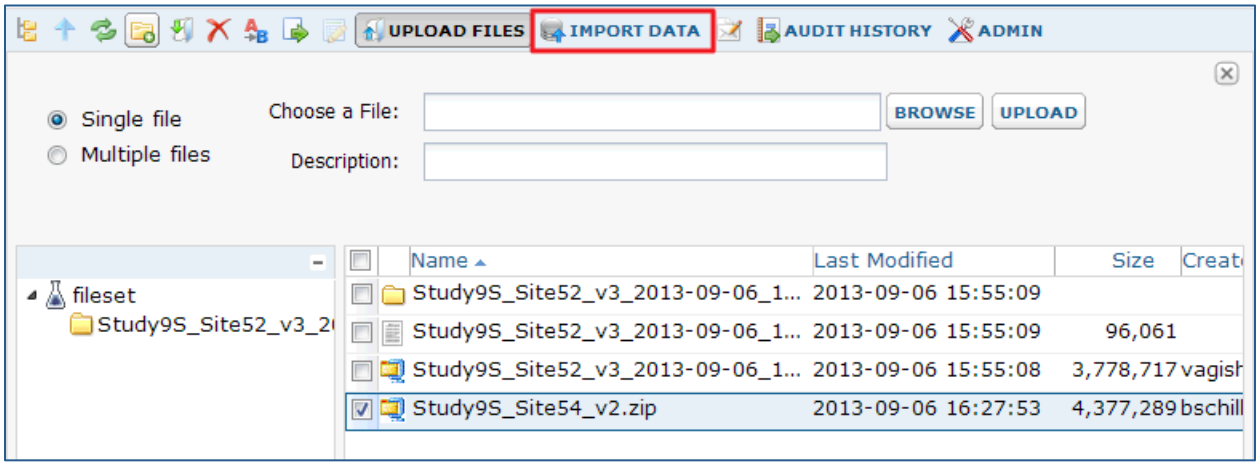


- In the **Files** browser, click **Upload Files**.
- Click the **Browse** button.
- Select Study9S_Site54_v2.zip that you just saved through Skyline.
- In the **Description** field enter 'Site 54'.
- Click the **Upload** button.



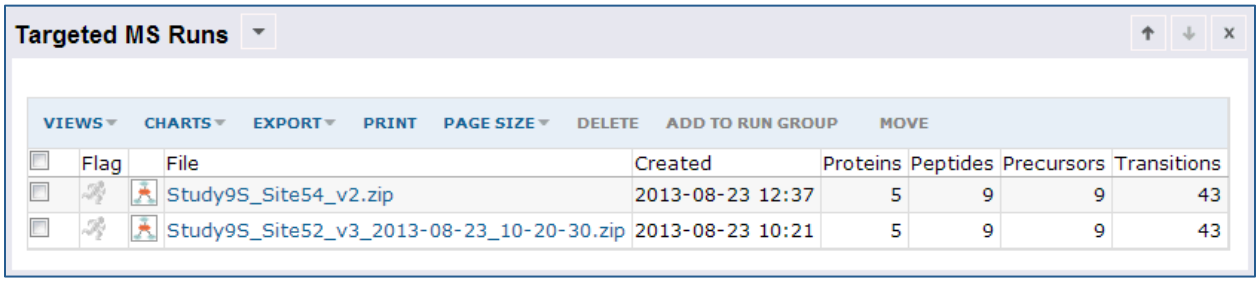
Once the file has been uploaded it will appear in the list of files in your folder.

- Check the box next to Study9S_Site54_v2.zip.
- Click **Import Data** in the toolbar, beside the **Upload Files** button**.**



- Click **Import** in the **Import Data** popup.

Wait for the file import to complete and then click the **Panorama Dashboard** tab in the upper right corner. You will now see 2 files (from sites 52 and 54) available under **Targeted MS Runs**.



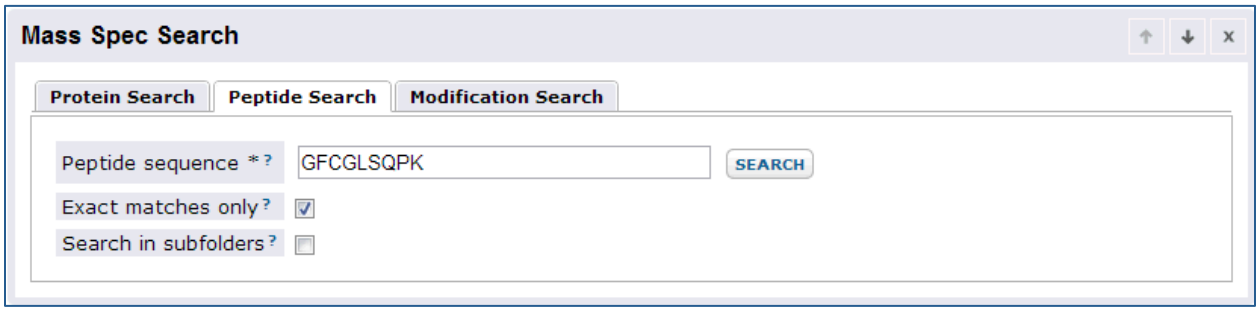
Search features in Panorama

Panorama allows you to search for proteins, peptides and even peptides with specific modifications across all the documents contained in a folder and its sub-folders. These search features are particularly useful for large projects and data sets, and help to compare results between data acquired over a long period of time.

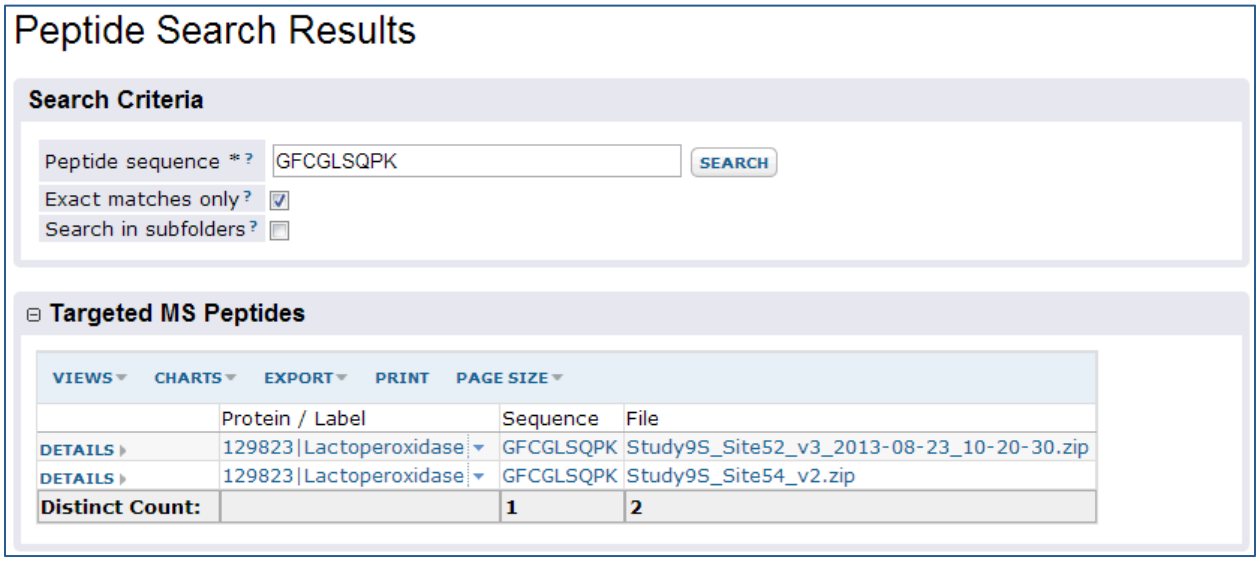
On the 'system suitability' folder page look at the **Mass Spec Search** box. You will see three available search options under the tabs **Protein Search**, **Peptide Search** and **Modification Search**. You will use the modification search option later in the tutorial. To perform a peptide search do the following:

- Click the **Peptide Search** tab.

- Enter 'GFCGLSQPK' in the **Peptide sequence** field.
- Check **Exact matches** only.
- Click the **Search** button.



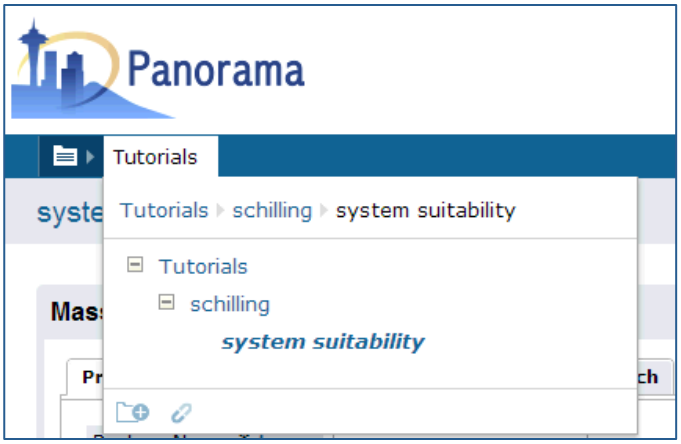
On the results page, in the **Targeted MS Peptides** box, you will see a list of documents in your folder where this peptide was observed. This peptide was found in both documents uploaded (sites 52 and 54) to this folder. You can view the details for the peptide in a document by clicking on the peptide sequence.



Sharing data in Panorama

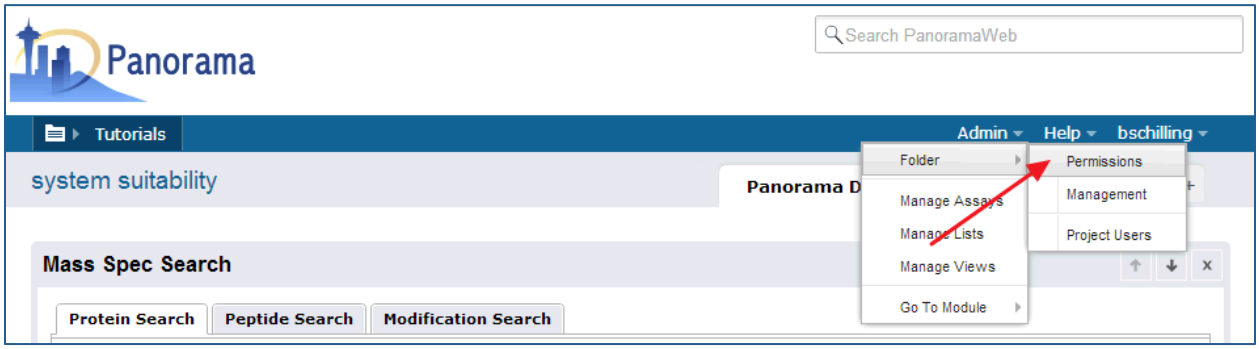
Panorama allows you to set fine-grained access control on your folders. You can grant access to your folders to individual collaborators or groups of researchers, and you can also configure the type of access (read-only, read and edit etc.).

To change the permissions on the 'system suitability' folder, first, make sure that you are on the 'system suitability' folder page. If not, hover over your project name (e.g. Tutorials) in the menu bar below the Panorama icon and click the 'system suitability' folder as shown in the image below.

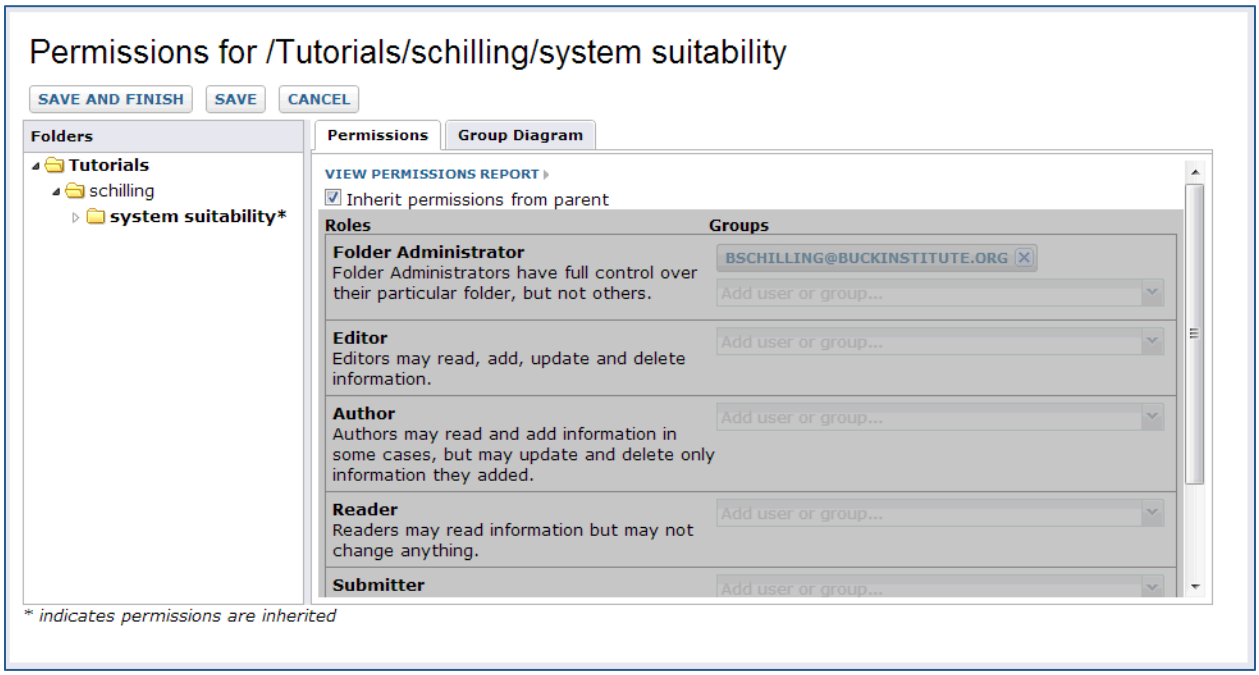


To change the permissions on this folder, navigate to the **Permissions** page by doing the following:

- On the menu bar, choose **Admin**, then **Folder** and click **Permissions**.



If you did not configure the permissions for this folder during the folder creation process, the permissions page should look like this.



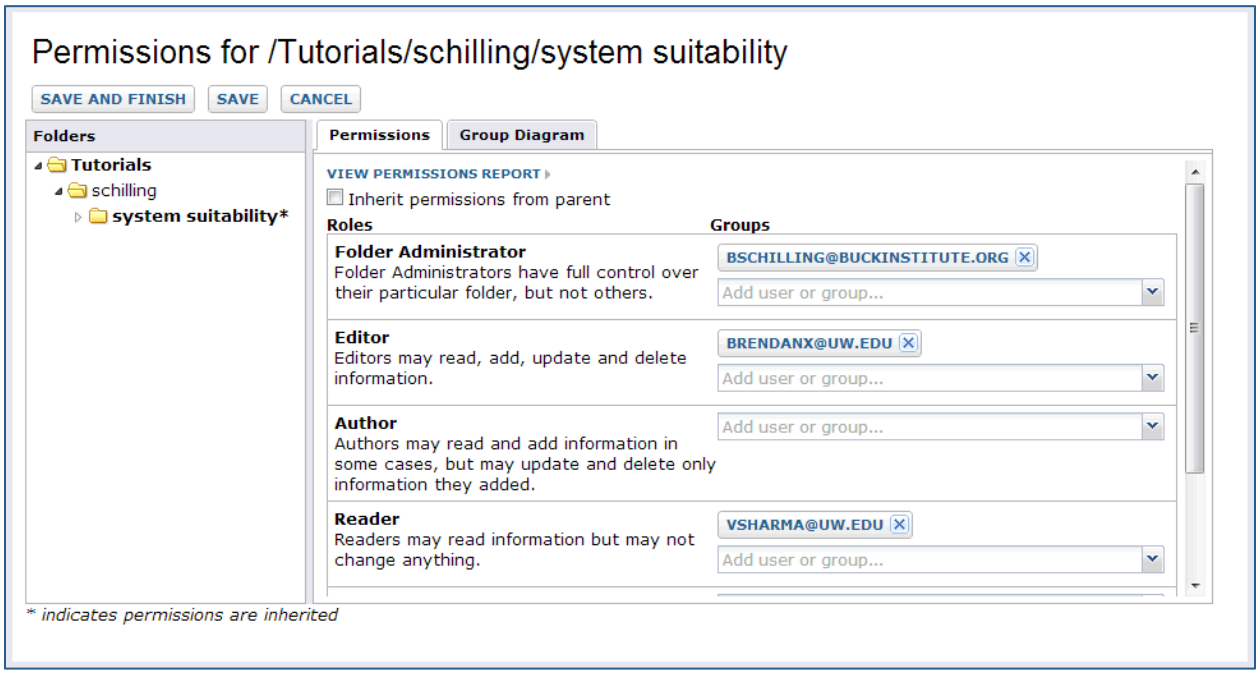
The email address selected for the **Folder Administrator** role should be the email address with which you requested a project on panoramaweb.org. The Folder Administrator has full control over a folder. To add Brendan MacLean (Skyline team) to the Editor role, which will give him the ability to add and delete documents to this folder, follow these steps:

- Uncheck **Inherit permissions from parent**, if it is checked.
- Select 'brendanx@uw.edu (Brendan MacLean)' from the combo-box next to **Editor**.
- Click the **Save** button**.**

To add Vagisha Sharma (Panorama team) to the Reader role:

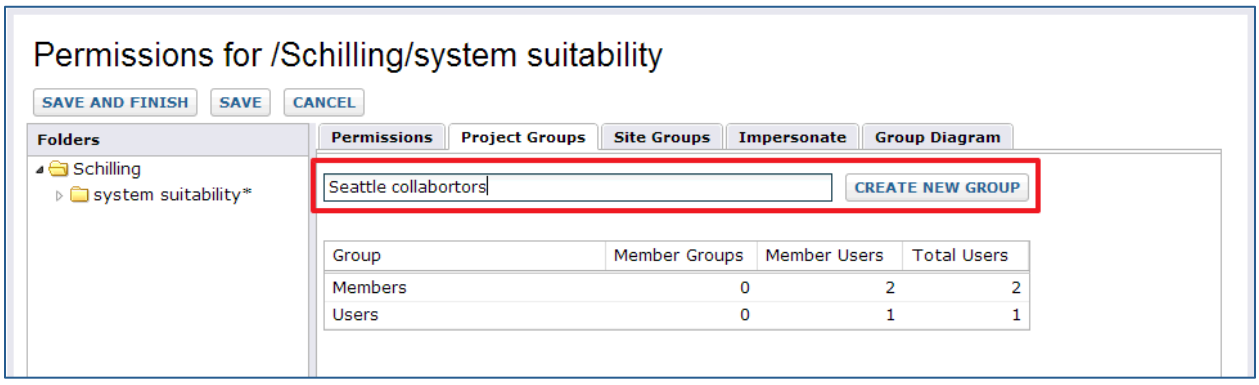
- Select 'vsharma@uw.edu' from the combo-box next to **Reader**.
- Click the **Save** button

The Reader role allows users to view data in a folder but they may not add or delete documents. The permissions page should now look like this:

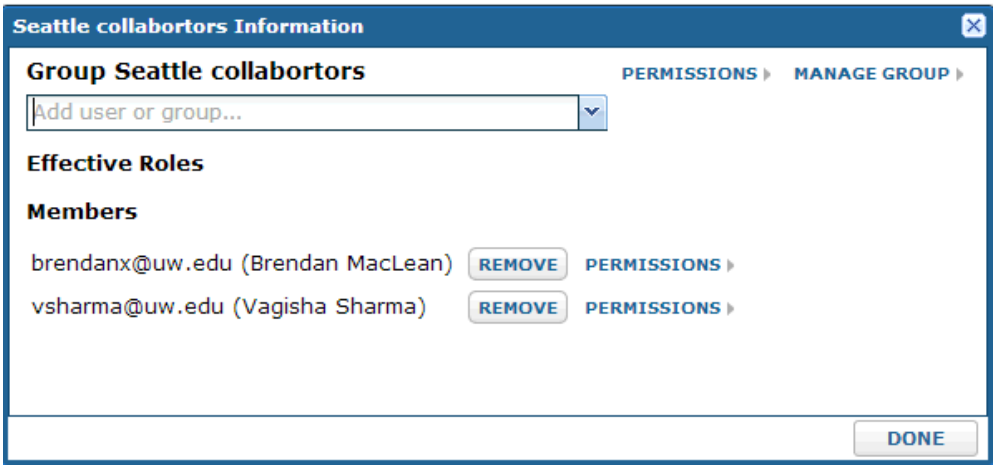


Instead of adding individual users to a role you can create a group of users and assign a role to the group. Only Project Administrators have permissions to create user groups. If you are working in your folder in the "Tutorials" project on panoramaweb.org, you do not have project administrator privileges. However, if you are working through this tutorial in your own project on panoramaweb.org or any other Panorama server, follow these steps to create a 'Seattle collaborators' group and assign it to the Reader role:

- On the Permissions page click on the **Project Groups** tab.
- In the **New group name** field enter 'Seattle collaborators' and click on **Create New Group.**



- In the information popup select Brendan and Vagisha from the pull down list to add them to the group.
- Click the **Done** button.

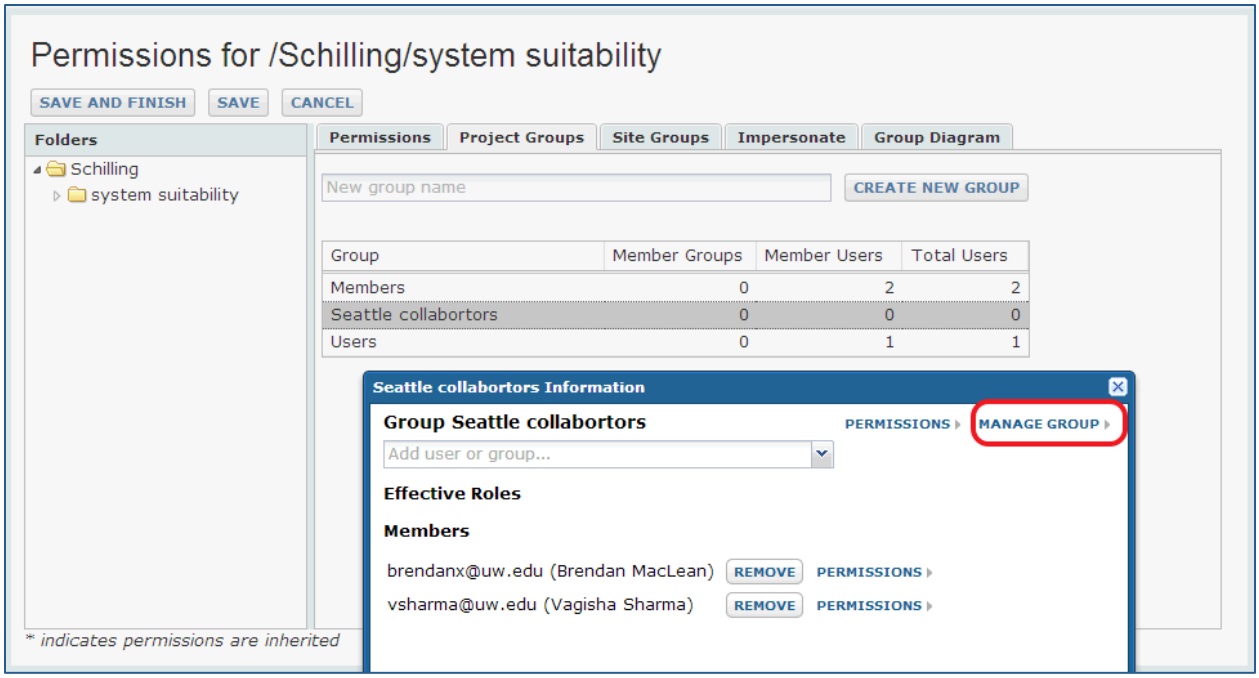


- Select the **Permissions** tab.
- Add the new 'Seattle collaborator' group to the **Reader** role.
- Click the **Save** button.
- Select the 'system suitability' folder in the folder navigation column.
- Add the 'Seattle collaborator' group to the **Reader** role for this folder.
- Click the **Save and Finish** button.

Members of the 'Seattle collaborator' group will be able to view the 'system suitability' folder if they have a direct link to the folder. They will not be able to view the parent project (e.g. Schilling) on the home page or navigate to the 'system suitability' folder through the folder navigation UI. To access a folder from the UI, users or user groups require read permissions not only on the folder but also on the parent folders all the way up to the root project folder. To enable the 'Seattle collaborator' group to access the 'system suitability' folder via the navigation UI, assign the **Reader** role to this group in the parent project folder (e.g. Schilling).

You can add more users later to the 'Seattle collaborator' group. All users in this group will have read access to the 'system suitability' folder. If you would like to add a user to the 'Seattle collaborator' group that does not already have an account on Panorama, you can do that by following these steps:

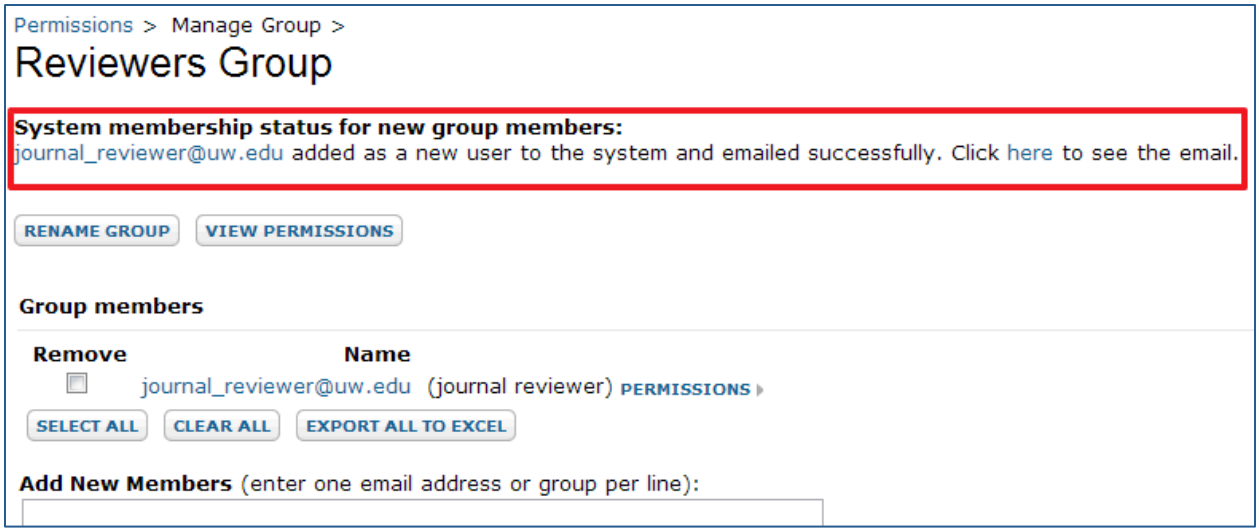
- Go back to the Permissions page.
  (On the menu bar, choose **Admin**, then **Folder** and click **Permissions**.)
- Click on the **Project Groups** tab.
- Click on the 'Seattle collaborators' group
- In the popup that appears click the **Manage Group** link in the top right corner.



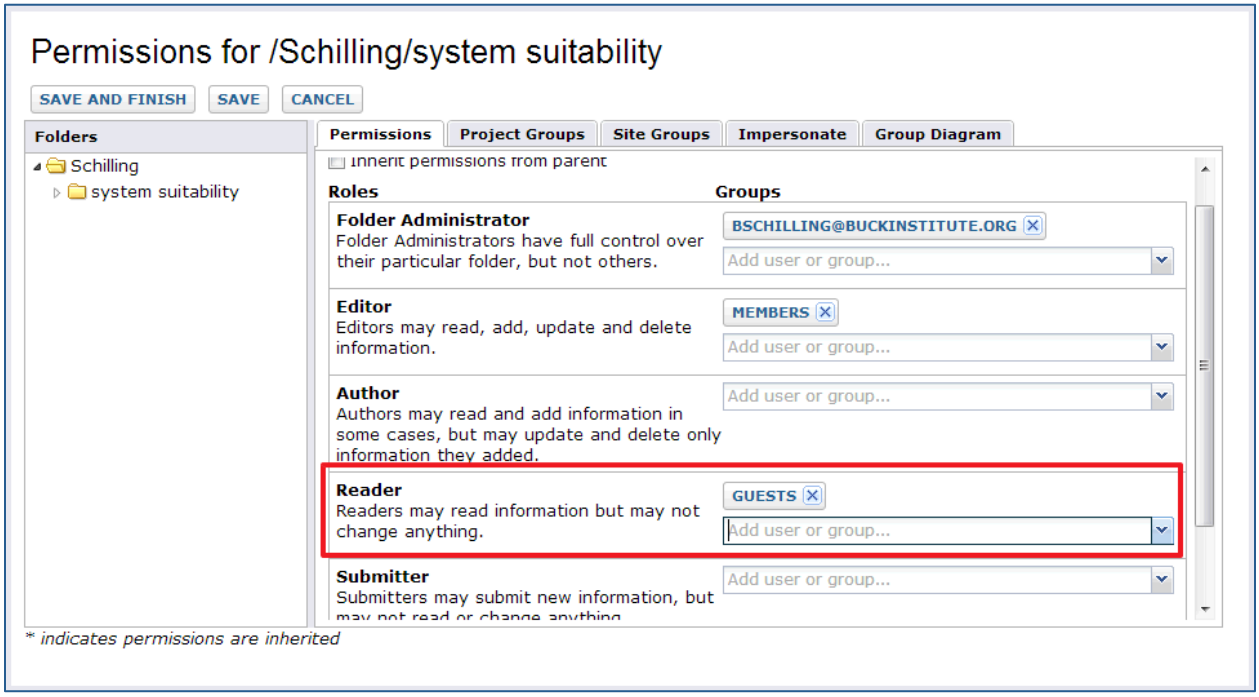
- On the Manage Group page, enter the email address of the new user in the **Add New Members** field.
- Click the **Update Group Membership**.

An email will be sent to the new user and they will be able login to view the data in the 'System suitability' folder once they setup a password for their account.

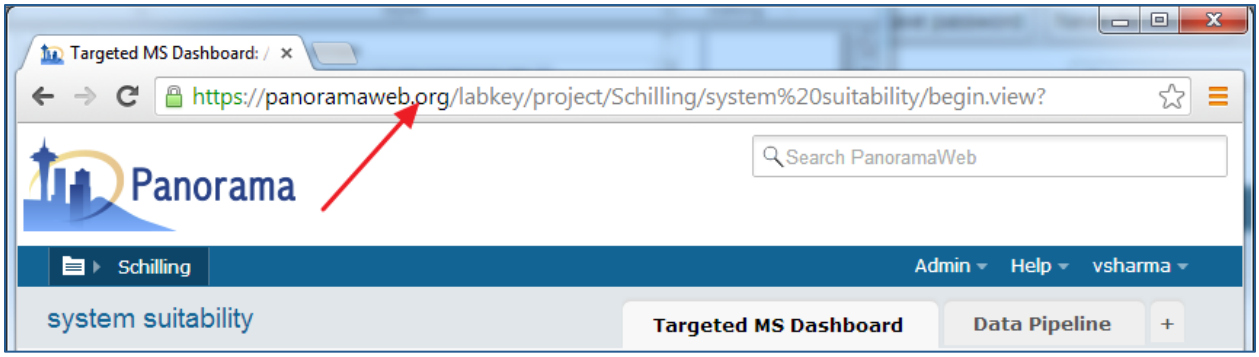
Panorama can be used, during a manuscript review process, to provide reviewers access to your data, so that they can view quantitative chromatograms and spectral library data as supplementary material to your paper. To do this, you can add a new user to an existing read-only group or create a new 'Reviewers' group and add it to the **Reader** role. This new user could be an email address that you have set up specifically for manuscript review, or you could use a fake email address. If you use a fake email address you can set the password for the account by clicking on a link to the email message that gets generated for the new user. This link will appear at the top of the **Manage Group** page when you update the group membership. You can provide this email address and password to the journal editor.



Once your paper has been accepted you can make the data public by adding **Site: Guests** to the **Reader** role, as shown below.



Anyone with a link to the folder that you made public will then be able to view it without requiring an account on panoramaweb.org. You can copy the link that you see in your browser's address or URL bar, when you are on the folder home page, and include it in your manuscript.



You can view the LabKey Server documentation on configuring permissions (https://www.labkey.org/wiki/home/Documentation/page.view?name=configuringPerms) for more information.

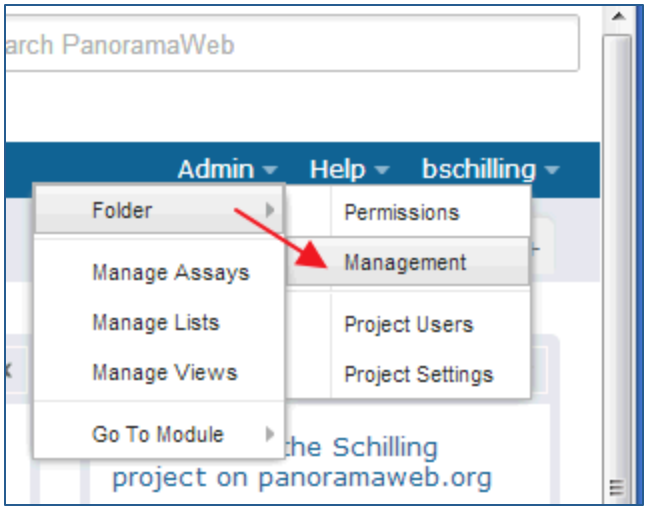
Viewing of PTM-containing peptides in Panorama

Many journals require visual display of MS/MS spectra of peptides containing post-translational modifications such as phosphorylation or acetylation. You can make use of Skyline to build spectral libraries from your peptide search results and view the annotated spectra in the Skyline Spectral Library Explorer. However, using the resulting Skyline document to satisfy journal requirements requires that you send the Skyline document to reviewers, and that they have a local installation of Skyline to view the document. Alternatively, you can publish the documents to Panorama, where reviewers can view the spectra in the Panorama spectrum viewer.

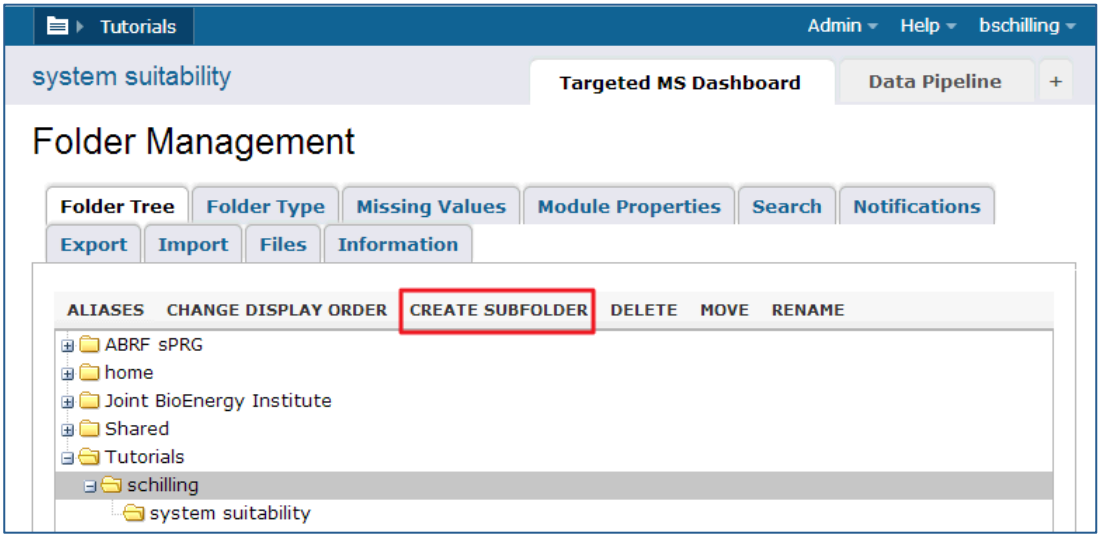
Next, you will import a Skyline document to Panorama that contains phosphorylated peptides, search for modified peptides and view their spectra.

Create a new folder in the 'Schilling' project called 'Phospho peptides', using another method for creating sub-folders in Panorama than you used before:

- In the upper right corner of the menu below the Panorama icon, click on **Admin.**
- From the drop-down menu choose **Folder** and click **Management.**



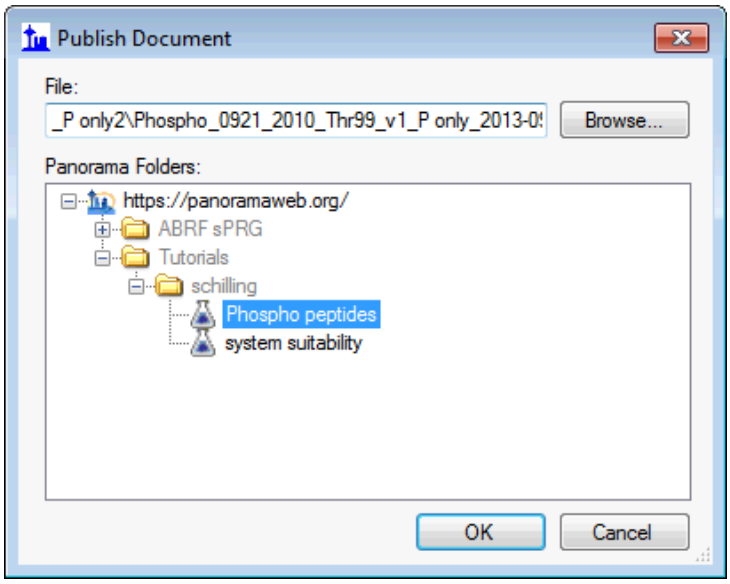
- On the **Folder Management** page, make sure that the **Folder Tree** tab is selected, and your folder (e.g. schilling) is highlighted.
- Click the **Create Subfolder** button.



- In the **Create Folder** wizard enter "Phospho peptides" in the **Name** text-field.
- Select **Panorama** folder type, as before.
- Click the **Next** button**.**
- For **Users/Permissions** keep the default selection: **Inherit from Parent Folder.**
- Click the **Next** button**.**
- Select **Experimental Data** on the **Configure Panorama Folder** page.
- Click the **Finish** button**.**

To add to the new folder a Skyline document containing a spectral library from a phosphorylation experiment, perform the following steps:

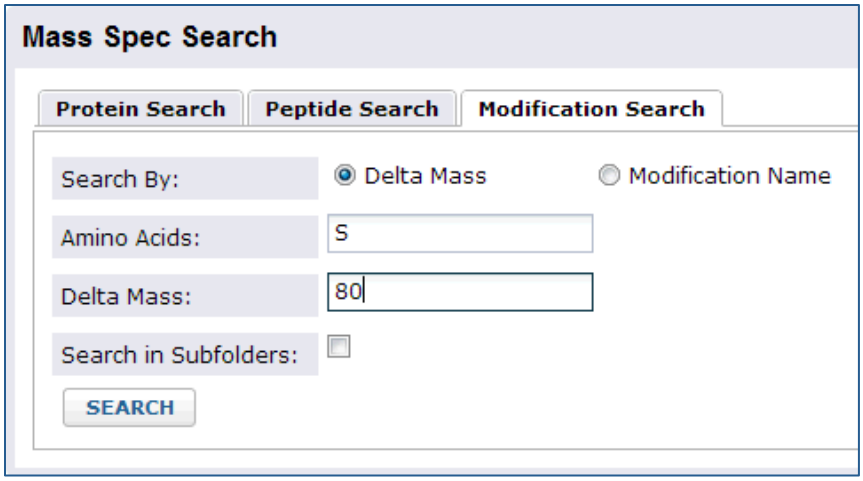
- In Skyline, open the file Phospho_0921_2010_Thr99_v1_P only.sky from the tutorial folder.
- Click the **Publish to Panorama** toolbar button. 
- Select the "Phospho peptides" folder from the folder tree in the **Publish Document** dialog.
- Click the **OK** button.



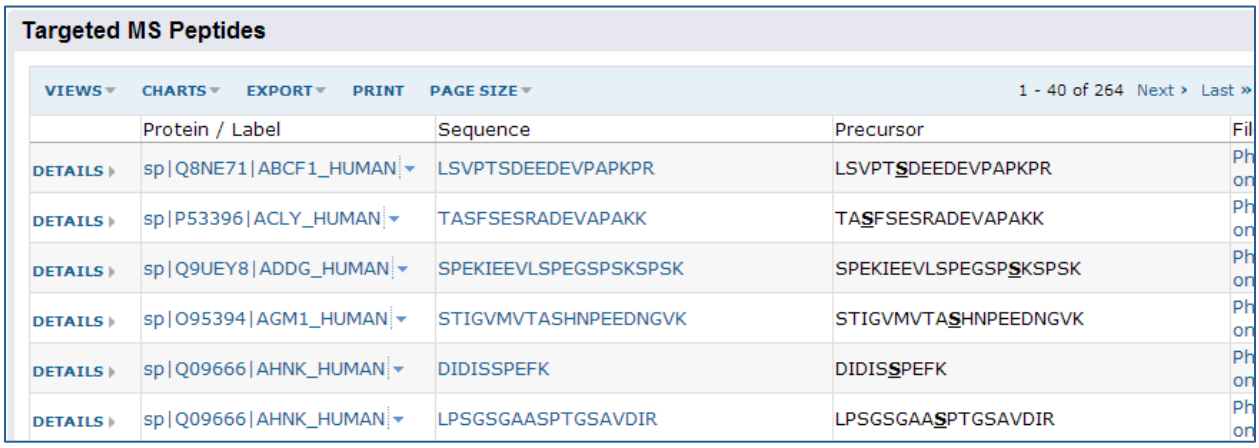
Wait for data import to complete and then go back to Panorama in your web-browser, and navigate to the "Phospho peptides" folder. Refresh the page (F5 in most browsers) if you are already on the "Phospho peptides" folder page. You should see the file that you just imported to Panorama in the **Targeted MS Runs** table. Click on the name of the file to see the document details. Clicking on any of the peptide sequences will take you to the peptide details page where you can view the library spectrum for the peptide. Click the back button to go back to the folder page.

To find all peptides in this document containing a phosphorylation, perform the following steps:

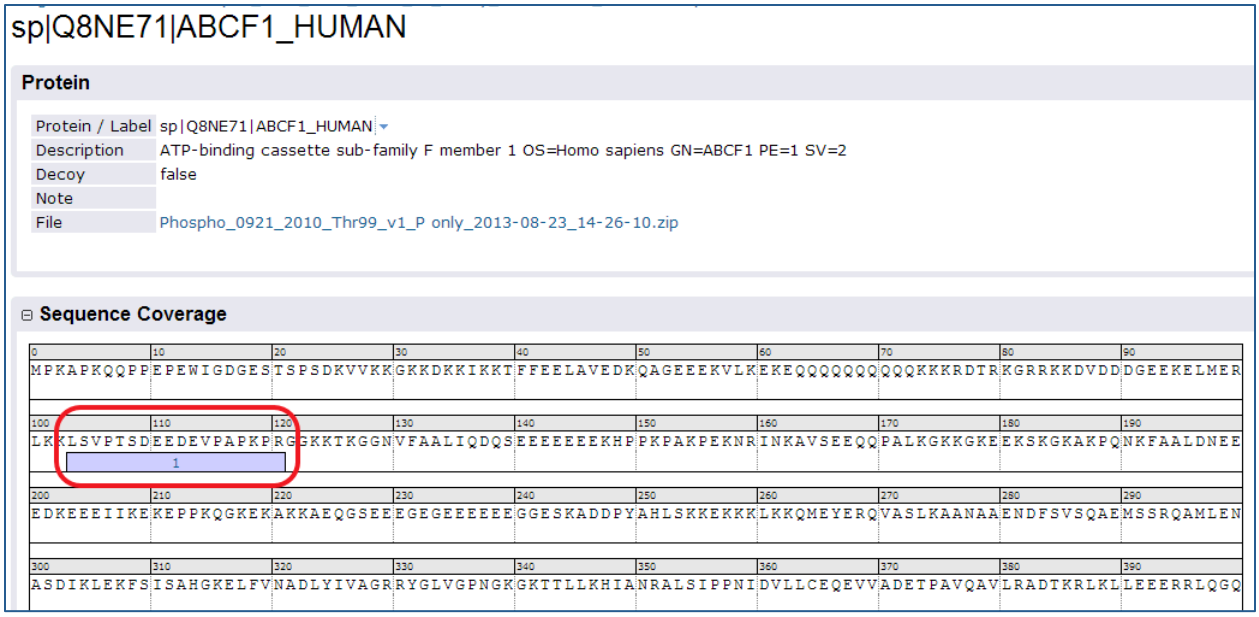
- In the **Mass Spec Search** box, select the **Modification Search** tab.
- Choose the **Delta Mass** option for **Search By**, if it is not already selected.
- Enter 'S' in the **Amino Acid** field and 80 in the **Delta Mass** field.
- Click the **Search** button.



You will now see a list of all the serine phosphorylated peptides in the document.



Click on the first peptide in the list, LSVPT**S**DEEDEVPAPKPR, to view its library spectrum. In the **Peptide Summary** box click on the name of the protein (sp|Q8NE71|ABCF1_HUMAN) to which this peptide belongs. You will be taken to the protein details page where you can see the location of this peptide in the protein sequence.



Conclusion

In this tutorial, you have been introduced to new ways you can share Skyline documents with collaborators, reviewers and the general public using Panorama, and the PanoramaWeb.org web site hosted by the MacCoss Lab at the University of Washington. You have requested a project on PanoramaWeb, registered an administrator account for that project with your email address, and created 2 new folders in that project. You have explored data from Skyline documents in these folders, searched for specific peptides and post-translational modifications, and customized access to the data in them. If you worked on this tutorial in your own project instead of the 'Tutorials' project, you can now just as easily go back to the folder management form and delete these tutorial folders to return your new project to its original state, ready for your own experimental data. We wish you luck in your research and hope that you find these tools useful in sharing and disseminating your findings.

Supplementary Note 2: A tutorial on building chromatogram libraries in Panorama

Panorama Chromatogram Libraries

Targeted assay development for proteins or peptides is typically a time-consuming and lengthy process. Creating an ideal set of peptides and transitions to measure for your proteins of interest requires careful experiment design and iterative optimization. Once an assay has been established, the assay parameters can be reused for designing future experiments. Chromatogram libraries in Panorama provide a way by which you can store targeted assays that have been curated in Skyline and reuse them in the future for measuring proteins and peptides in other samples, as well as share them with other researchers. Chromatogram libraries can also capture physiochemical and experimental properties such as the relative ion abundance, retention time, ion mobility, and optimal instrument parameters such as collision energy etc. These can be particularly relevant in guiding targeted experiment design.

In this tutorial we will

- Create a folder in Panorama for chromatogram library data.
- Add Skyline documents, with curated targeted results, to the chromatogram library folder.
- Download the chromatogram library from Panorama and use it in Skyline.

Getting started

To start this tutorial, download the following ZIP file:

https://panoramaweb.org/tutorials/PanoramaChromatogramLibraries.zip

Extract the files to a folder on your computer, like:

C:\Users\vsharma\Documents

This will create a new folder:

C:\Users\vsharma\Documents\PanoramaChromatogramLibraries

It will contain all the files necessary for this tutorial.

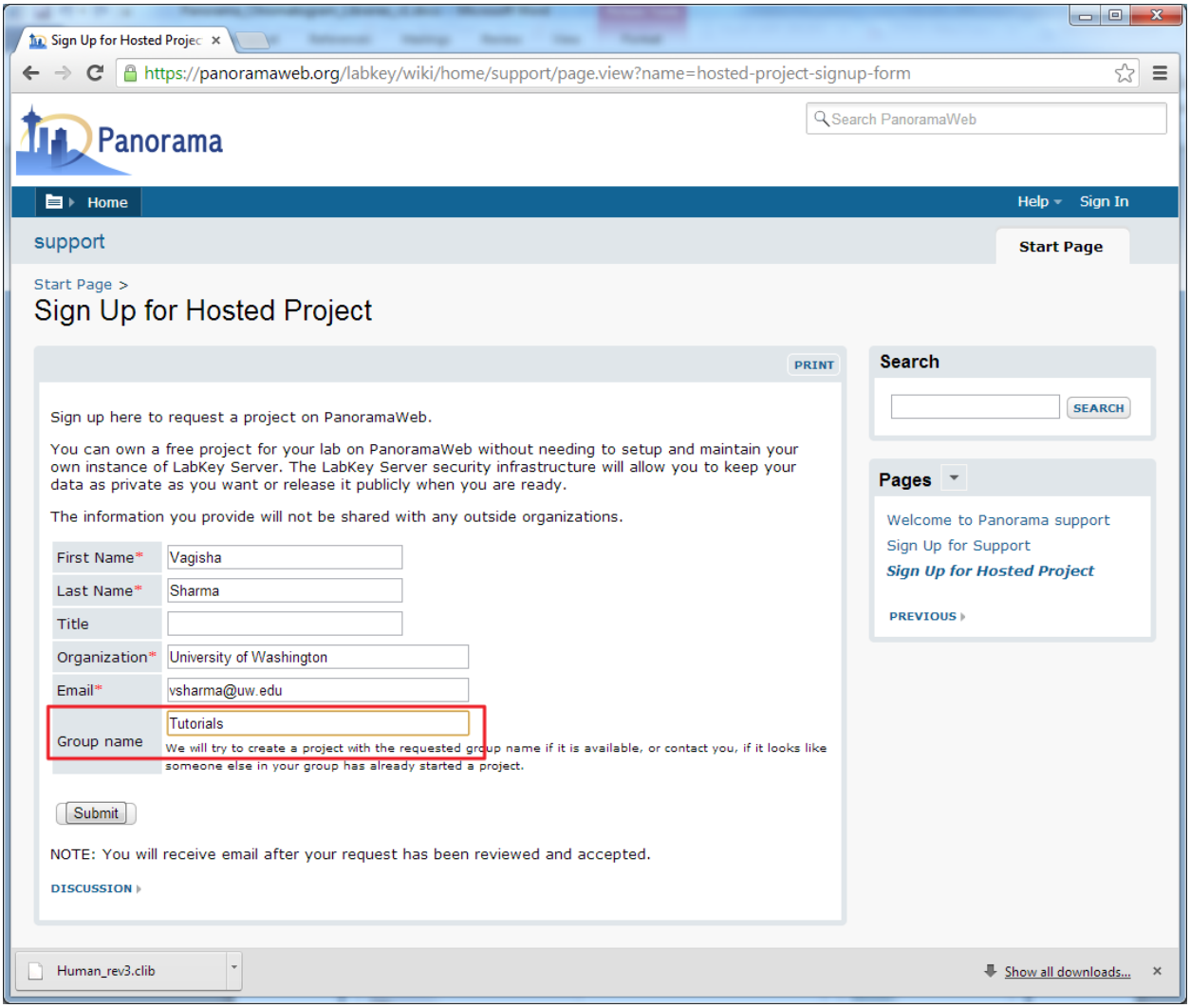
The data used in this tutorial was published in Stergachis *et al.*[1] where the authors described a high-throughput method for selecting optimal peptides and fragment ions for targeted proteomics applications. The Skyline files used in this tutorial contain assays for a subset of the 96 human transcription factors on which they demonstrated their approach.

Signing up for an account on panoramaweb.org

This tutorial assumes that you will be working in a sub-folder of the "Tutorials" project on panoramaweb.org, the Panorama server hosted by the MacCoss lab at the University of Washington. However, if you already have an existing account and project on panoramaweb.org, or have an account on another Panorama server you are welcome to use that, and skip to the next step.

To request a folder on panoramaweb.org for working through this tutorial, fill out the sign-up form on this page: https://panoramaweb.org/labkey/wiki/home/support/page.view?name=hosted-project-signup-form .

- Fill in the required fields, marked with a red asterisk, in the form shown below.
- Enter 'Tutorials' in the **Group name.**
- Click on the **Submit** button**.**

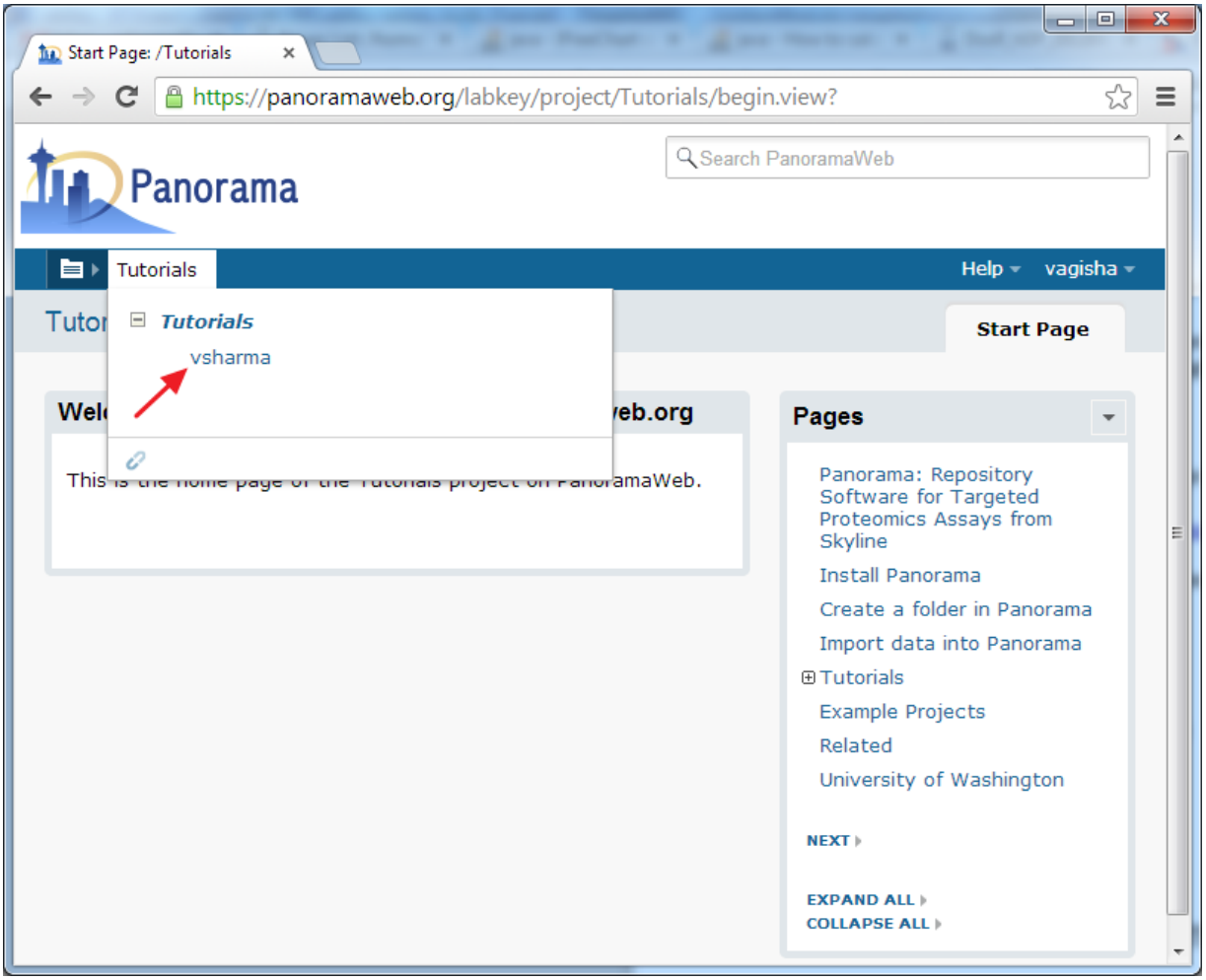


Once your request is approved, a folder with full administrative rights will be created for you in the "Tutorials" project on the panoramaweb.org. You will also receive a welcoming email with a link to setup a password for your account, and complete the registration steps.

Creating a chromatogram library folder in Panorama

Login to the Panorama server and navigate to your folder. If you are working in the "Tutorials" project on panoramaweb.org follow these steps:

- Click on the **Projects** tab to see a list of all the projects to which you have access on the server.
- Click on the "Tutorials" project.
- On the project homepage, hover over the project name in the menu bar below the Panorama icon and click on the folder that was created for you (e.g. 'vsharma').

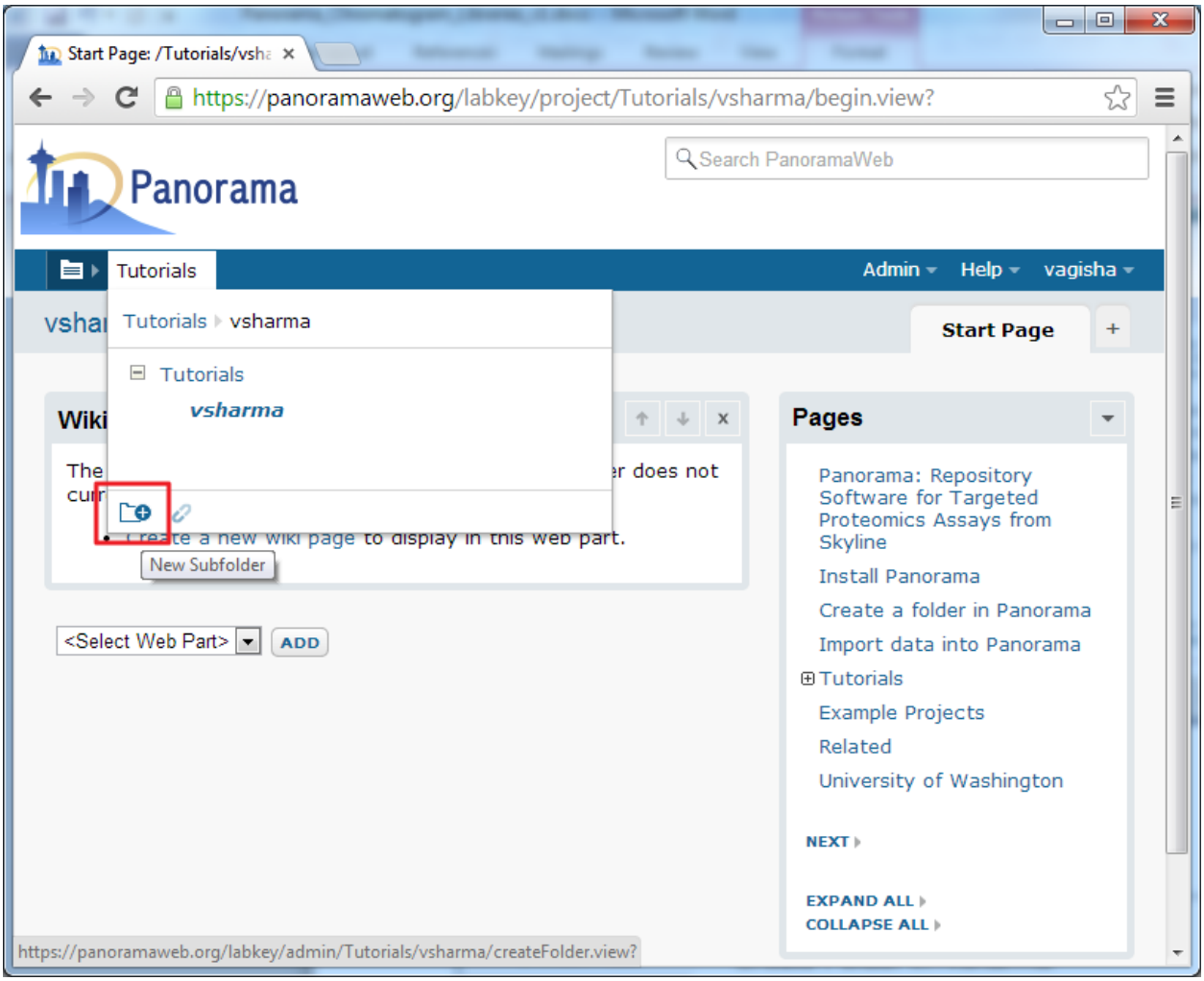


When organizing your data for chromatogram libraries, we recommend that you create a folder for each species that you study, and under each species folder you can create subfolders for the instrument type

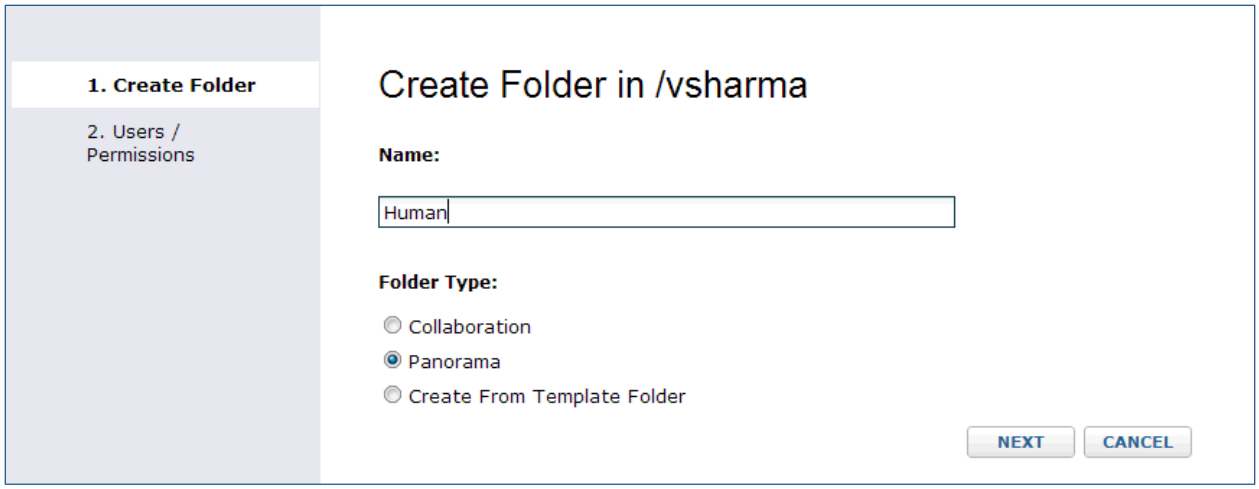
where the data was collected, or any other experimental conditions for which it is appropriate to have separate chromatogram libraries. In this tutorial you will create a subfolder for building a chromatogram library for human transcription factors.

To create a sub-folder for this tutorial, do the following:

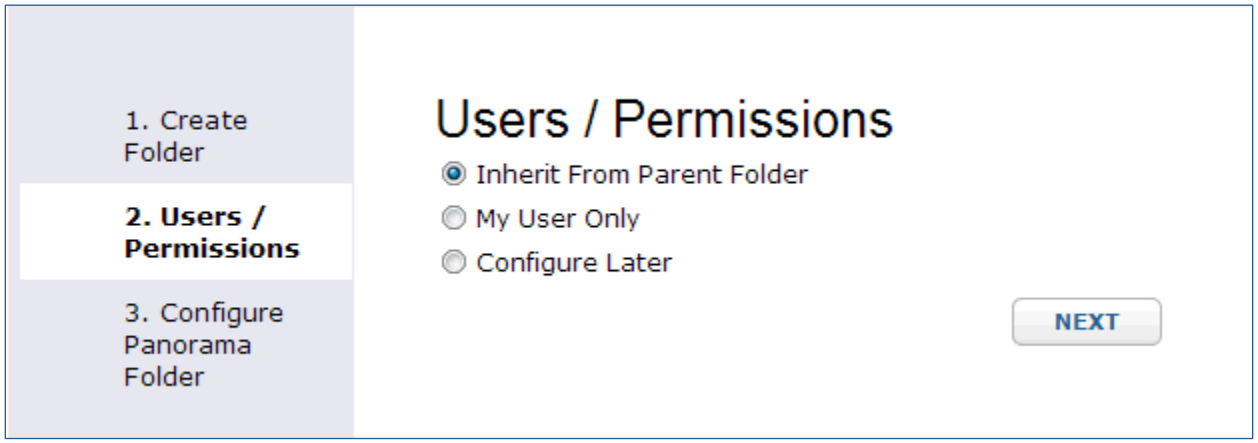
- Click on the new folder icon shown in the image below.



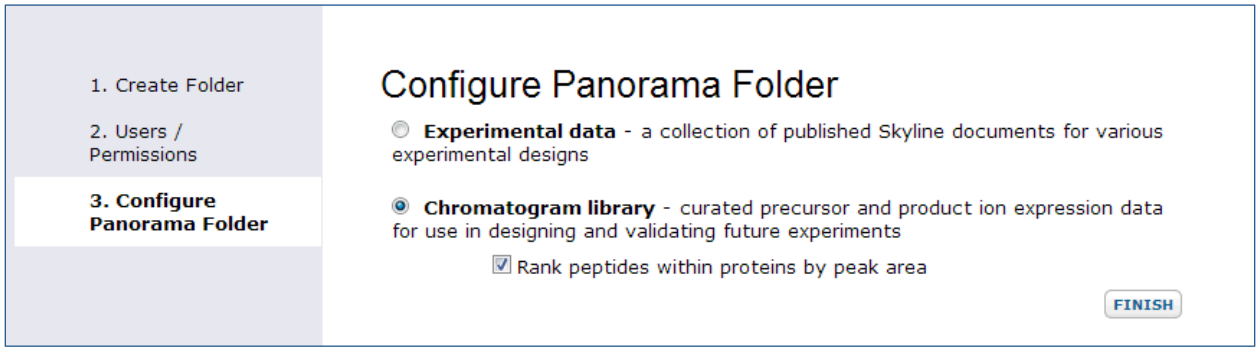
- In the **Create Folder** form, enter 'Human' in the folder **Name** field.
- Select the **Panorama** option under **Folder Type**, as shown below.



- Click on the **Next** button.
- On the **Users / Permissions** page accept the default selection.



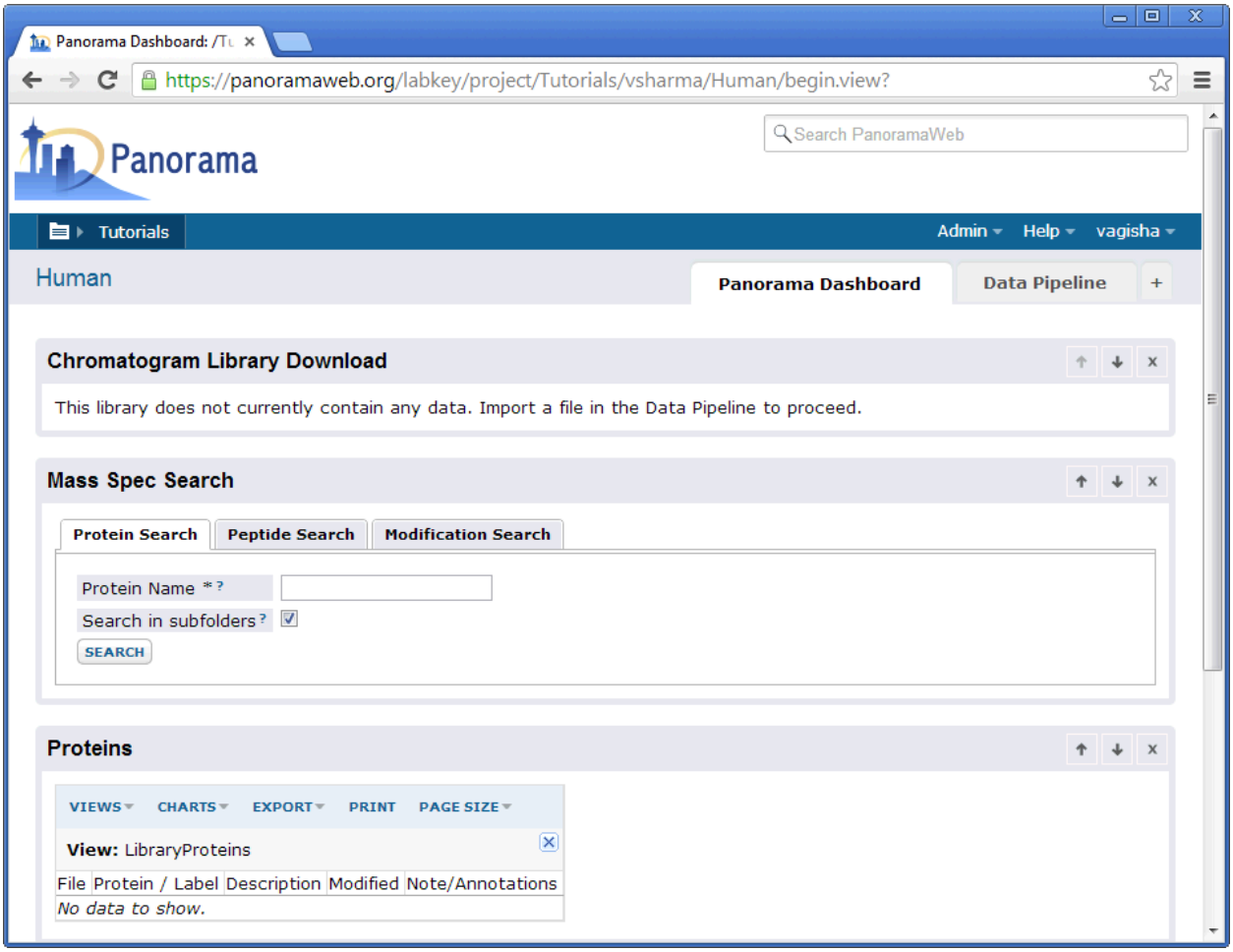
- Click on the **Next** button.
- On the **Configure Panorama Folder** page choose the **Chromatogram library** option.
- Check **Rank peptides within proteins by peak area**.



- Click on the **Finish** button.

The **Rank peptides within proteins by peak area** box should be checked when you have targeted results containing relative peptide peak areas for a given protein. Leave this box unchecked if you do not yet have quantitative data from proteolytically digested proteins, such as when you are working with synthetic peptides.

After successful folder creation, you will be taken to the home page of the 'Human' chromatogram library folder that should look as follows:



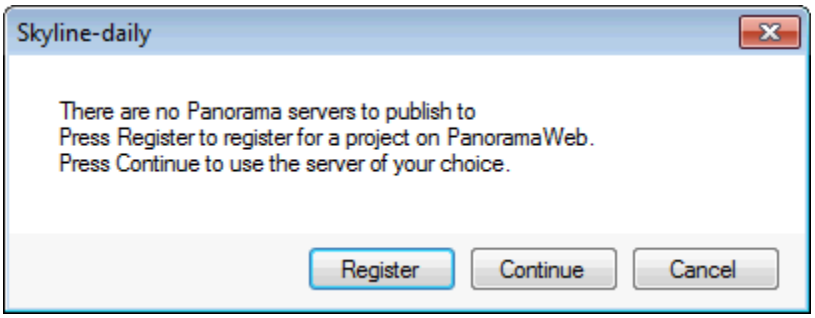
You are now ready to upload Skyline documents to this folder.

Publishing Skyline documents to a library folder in Panorama

Open the "Stergachis-SupplementaryData_2_a.sky" file in Skyline either by double-clicking on the file in Windows Explorer or by going through the **File > Open** menu in Skyline. This file contains peptides and fragment ions for 10 human transcription factors published in Stergachis *et al.*[1]. To publish this document to your library folder on Panorama, follow these steps:

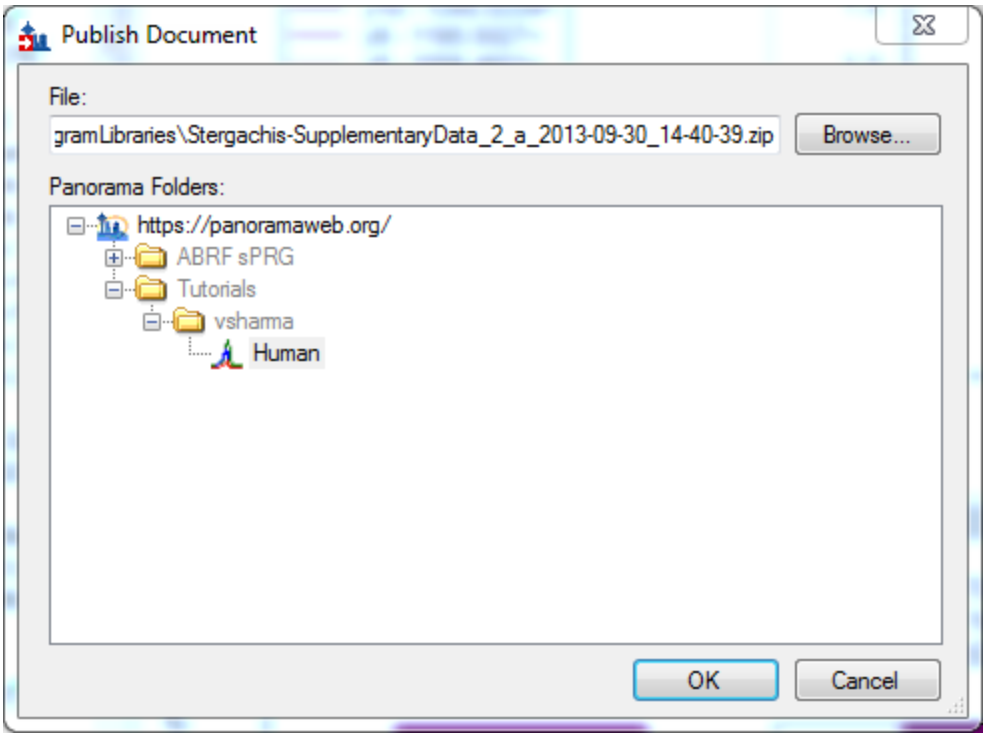
- Click on the Publish to Panorama button in the toolbar, shown in the image below. Alternatively, on the **File** menu, click on **Publish to Panorama**.

If you have not yet set up a Panorama server in Skyline, you will see the following message.



- Click on **Continue.**
- In the **Edit Server** form, the default value in the **URL** field is https://panoramaweb.org. This is the URL of the server hosted at the University of Washington, and the server where you have an account if you followed the registration steps at the beginning of this tutorial. If you are working on a server other than panoramaweb.org, enter the URL of your server instead.
- Enter your email address and password on the Panorama server.
- Click on **OK**.

Skyline will now display a form with the directory tree on your Panorama server. Chromatogram library folders will have the chromatogram icon next to them.

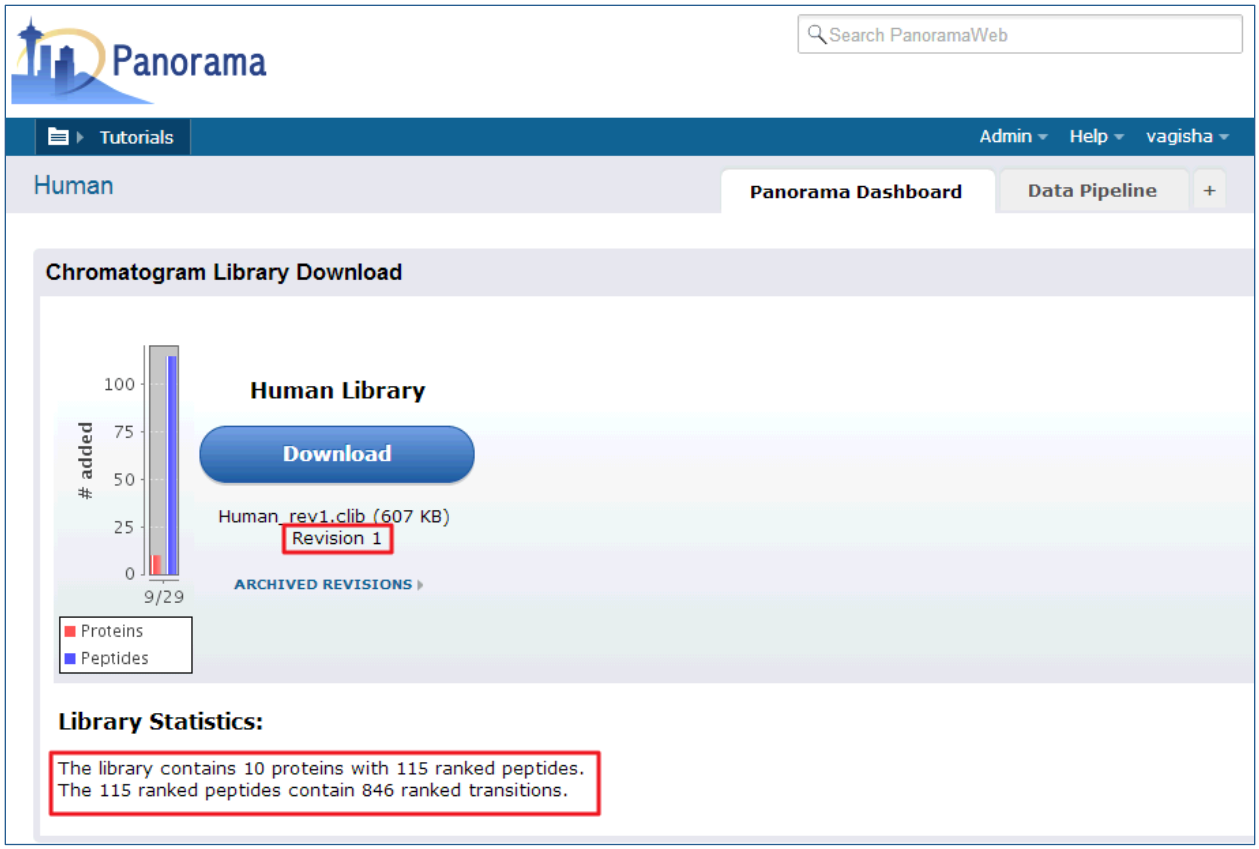


- Navigate to and select the 'Human' folder that you just created in Panorama.
- Click the **OK** button.

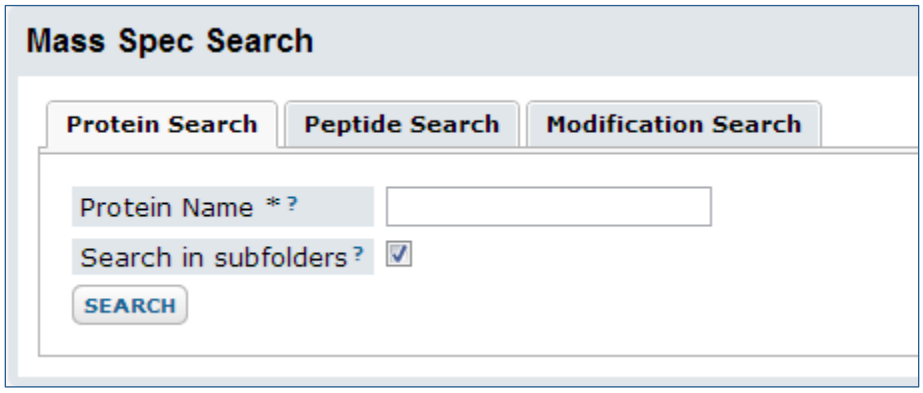
Skyline will upload a ZIP archive of your document to the selected library folder on the Panorama server.

Viewing chromatogram library data in Panorama

Once your document has been imported into Panorama, go back to the web-browser where the home page of the 'Human' library folder should still be open. Refresh the page (F5 in most browsers) and, in the **Chromatogram Library Download** box, you will see that the first version (Revision 1) of your 'Human Library' is now available for download. A brief summary of the contents of the library is also displayed under the **Library Statistics** heading.



Further down on the page, in the **Mass Spec Search** box, you can search for proteins, peptide sequences or peptides with specific modifications in your library.



The tables below the **Mass Spec Search** box display a list of the proteins and peptides in the library folder. Clicking on an entry in one of these tables will display more details for the selected protein or peptide. Click on 'CTCF' in the **Proteins** table to bring up a page with more details for this protein. The

Sequence Coverage box displays the sequence, with colored boxes highlighting the peptides for which there are measurements in the library.

The **Peptides** table displays a list of these peptides along with some information that was contained in the uploaded Skyline document, such as the retention time.

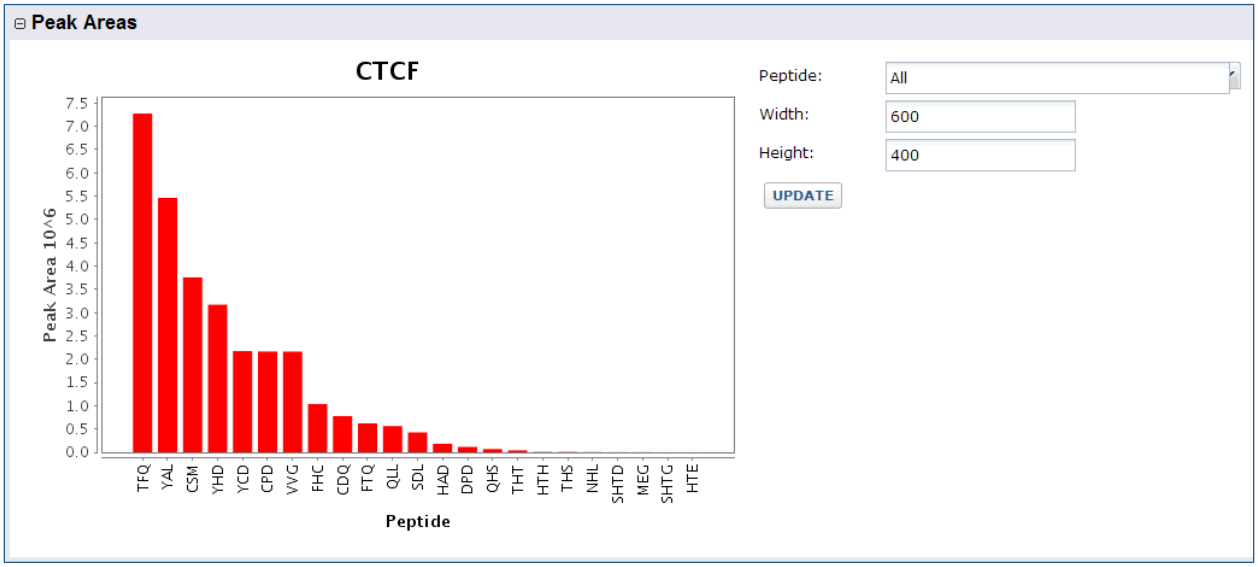
Peptides

VIEWS CHARTS EXPORT PRINT PAGE SIZE 1 - 23 of 23

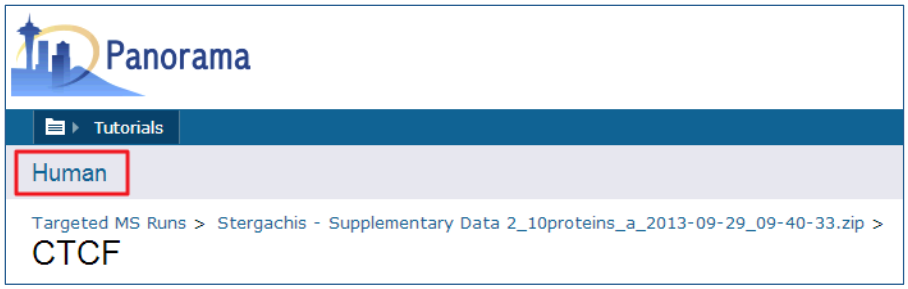
	Peptide	Peptide Neutral Mass	Missed Cleavages	Rank	Avg. Measured RT	Predicted RT	RT Score
DETAILS	MEGDAVEAIVEESETFIK	1995.9347	0		24.9907		
DETAILS	DPDYQPPAK	1029.4767	0		25.5342		
DETAILS	VVGNMKPPKPTK	1294.7432	0		24.8102		
DETAILS	TFQCELCSYTCPR	1720.7008	0		37.8053		
DETAILS	SHTDERPHK	1105.5265	0		24.3577		
DETAILS	NHLNTHTGTRPHK	1511.7705	0		46.2283		
DETAILS	CPDCDMAFVTSGELVR	1855.7903	0		42.3704		
DETAILS	HTHEKPFK	1022.5298	0		24.7994		
DETAILS	CSMCDYASVEVSK	1534.6102	0		34.4654		
DETAILS	SHTGERPFQCSLCSYASR	2141.937	0		24.7531		
DETAILS	THSGEKPYECYICHAR	2006.8727	0		45.1026		
DETAILS	FTQSGTMK	898.4219	0		24.8384		
DETAILS	HTENVAK	797.40314	0		24.7963		
DETAILS	FHCPHCDTVIAR	1511.6761	0		26.5643		
DETAILS	SDLGVHLR	895.48755	0		28.8680		
DETAILS	QHSYIEQGK	1088.525	0		24.7637		
DETAILS	YCDAVFHER	1195.508	0		28.5533		
DETAILS	YALIQHQK	999.5502	0		25.4094		
DETAILS	CDQCDYACR	1246.4165	0		25.0130		
DETAILS	THTGEKPYACSHCDK	1789.7512	0		24.6605		
DETAILS	QLLDMHFK	1030.527	0		36.4861		
DETAILS	YHDPNFVPAAFVCSK	1750.8137	0		38.9469		
DETAILS	HADNCAGPDGVEGENGGETK	2012.813	0		24.8724		

VIEWS CHARTS EXPORT PRINT PAGE SIZE 1 - 23 of 23

Below the **Peptides** table is a bar graph that displays the peak areas of the peptides, ordered from the highest to the lowest measured peak area



Go back to the home page of your library folder either by clicking on your browser's back button or by clicking on the folder name ('Human') above 'CTCF' as shown in the image below.

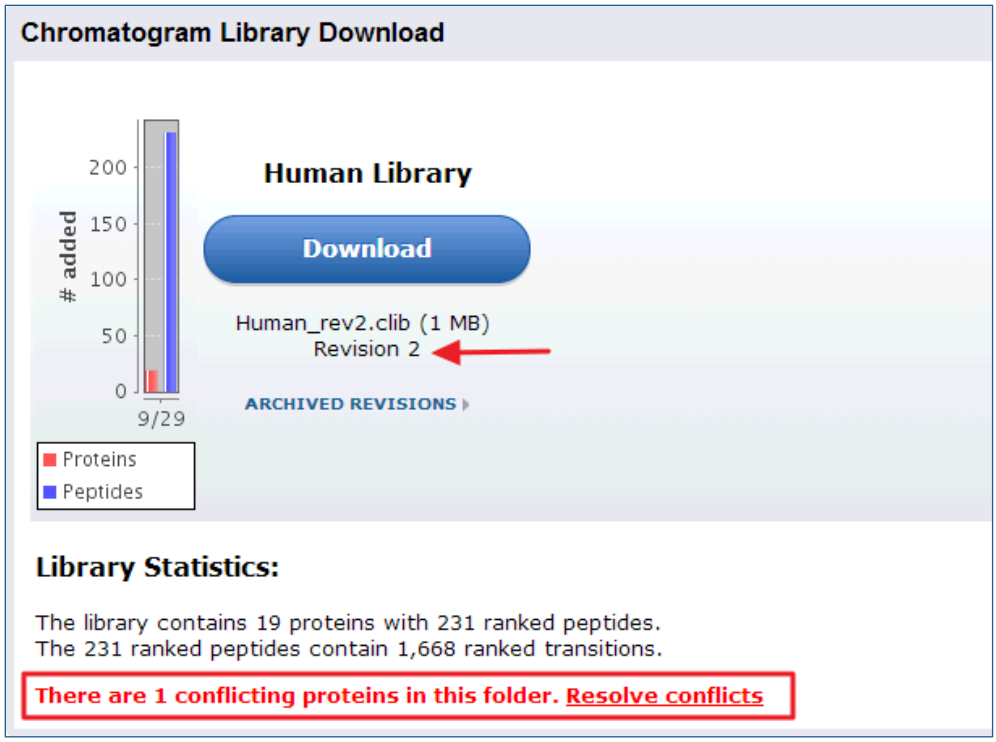


Updating chromatogram libraries

As you develop assays for more proteins or peptides, you can publish Skyline documents containing those assays to the same library folder. Panorama will build a new version of the library each time a Skyline document is imported to the folder. Any proteins that were not already included in the library will be added.

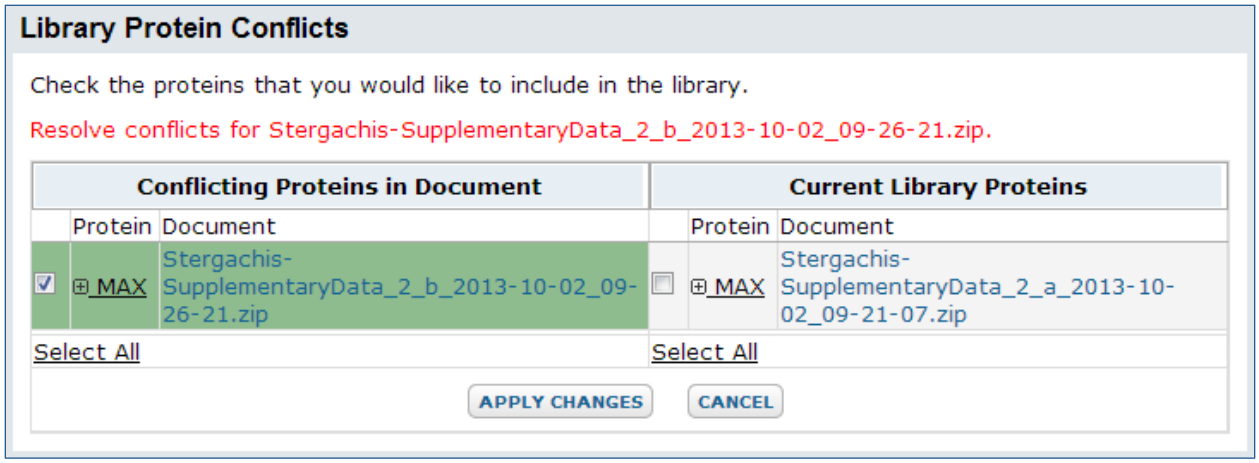
Open the file "Stergachis-SupplementaryData_2_b.sky" in Skyline. This file contains another subset of transcription factor assays from *Stergachis et al.*[1]. This file also contains measurements for the transcription factor MAX that was also included in the first document that you published to the library folder. Two additional peptide measurements are included for this protein in this document. Publish this document to the 'Human' chromatogram library folder on Panorama by clicking on the **Publish to Panorama** button in the Skyline toolbar and selecting the 'Human' folder in the Panorama directory tree. Wait for the file import to complete, and then go to your browser and refresh the home page of the library folder. You will see that a new version of the library is now available in Panorama, as indicated by the higher revision number (2) in the **Chromatogram Library Download** box. This library includes the new proteins, peptides and transitions from the Skyline file that you just uploaded, reflected in the updated library statistics.

You should also see a warning message below the library statistics about a conflicting protein. This message is displayed because the document you just uploaded contains a protein (transcription factor MAX) which already existed in the library.

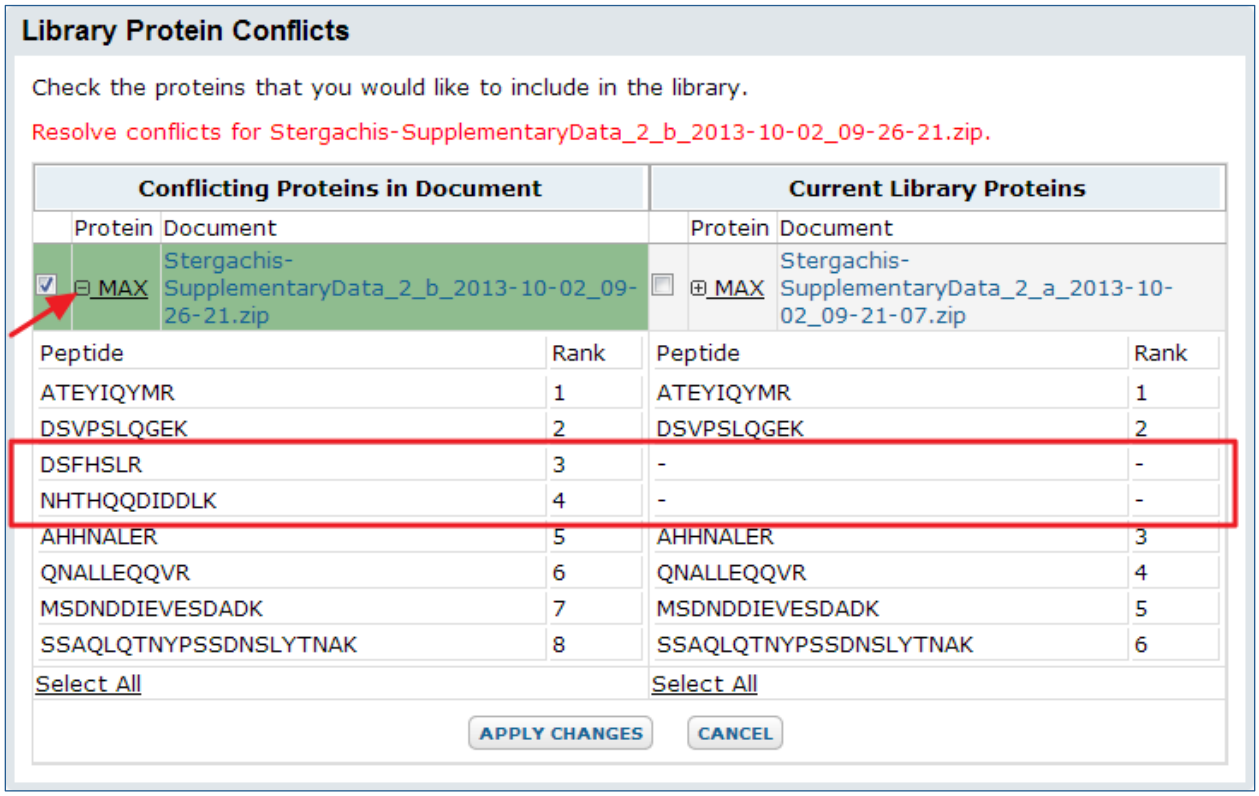


In case of a conflict, Panorama marks the incoming version of the protein as conflicted, and continues to include the older version of the protein in the library, till the conflict is resolved. The library built after uploading "Stergachis-SupplementaryData_2_b.sky" still contains peptides, transitions and chromatograms for protein MAX from the first document that was uploaded to the library folder. To tell Panorama to use the newer version of the protein click on the **Resolve conflicts** link.

You will now see a table containing a list of conflicting proteins displayed side by side. The left column, titled **Conflicting Proteins in Document**, displays the version of the protein contained in the document that you just uploaded. The right column, titled **Current Library Proteins** has the version contained in the current library.



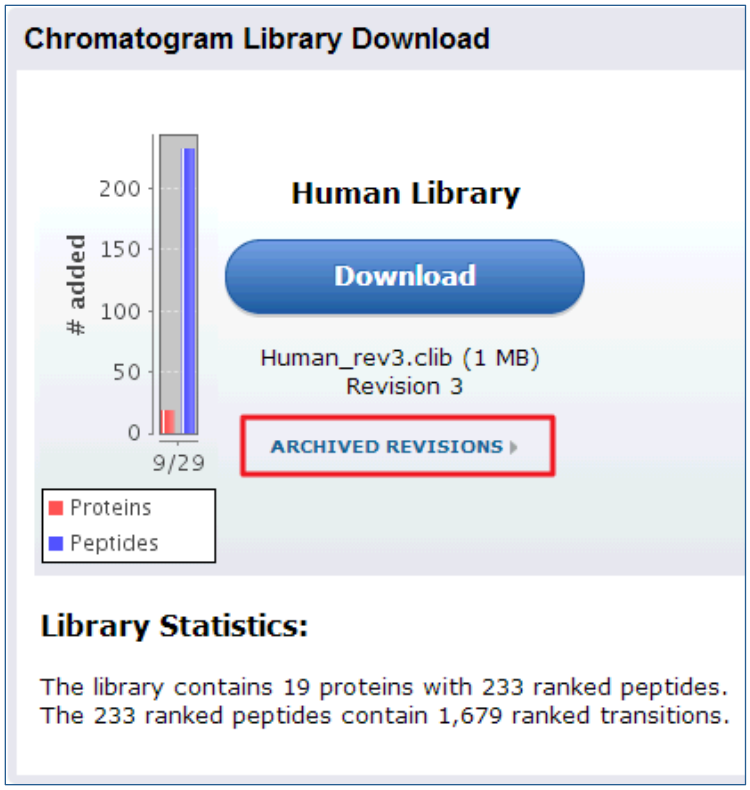
Click on the [+] icon next to the protein name to view the list of peptides measured for this protein in the two versions. The new document contains measurements for two additional peptides that are marked in the image below.



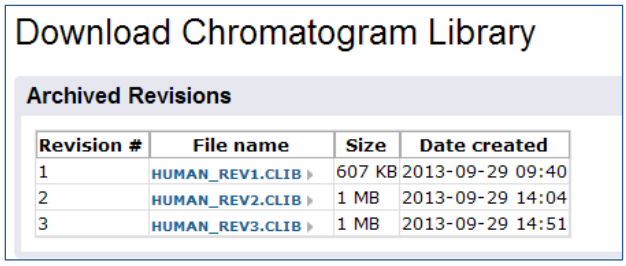
Select the new version of the protein by checking the box next to the protein name in the left column (**Conflicting Proteins in Document**) and click on the **Apply Changes** button. Panorama will now rebuild the library and include the new version of the protein with all its peptides and transitions. Any peptides or transitions that existed only in the older version of the protein will be removed from the library. Once the new library has been built you will be taken to the home page of the library folder where you will see that a new revision (3) of the library is available. The conflict message should no longer be displayed.

Click on the **Download** button and save the chromatogram library file (Human_rev3.clib) to your computer. You will use this library in the next section of the tutorial.

You can access older revisions of the library by clicking on the **Archived Revisions** link below the library file name, shown in the image below.



A table with all the library revisions will be displayed. You can download older revisions by clicking on a file name in the **Archived Revisions** table.



Using chromatogram libraries in Skyline

You will now prepare a new Skyline document to measure the proteins CTCF and MAX in another sample. The library you just downloaded from Panorama contains chromatograms for peptides from these proteins. You will use the library to filter the set of peptides and transitions in your Skyline document to the best responding ones contained in the library. You will also see how you can compare fragment ion or transition chromatograms obtained in a new experiment with chromatograms contained in the library to confirm peptide identifications.

Before you begin, make sure that the peptide and transition settings in Skyline are configured correctly. Open a new Skyline document by selecting **New** from the **File** menu.

Configure the peptide settings as follows:

- Click on **Peptide Settings** in the **Settings** menu.
- Click on the **Digestion** tab.
    - Select “Trypsin [KR | P]” from the **Enzyme** dropdown list.
    - Select 0 **Max Missed Cleavages**.
    - Select **None** from the **Background proteome** dropdown list.
- Click on the **Filter** tab.
    - Enter ‘7’ in the **Min length** textbox.
    - Enter ‘23’ in the **Max length** textbox.
    - Enter ‘0’ in the **Exclude N-terminal AAs** textbox.
    - Uncheck **Exclude potential ragged ends**.
    - Uncheck any selected options in the **Exclude peptides containing** list.
    - Check **Auto-select all matching peptides**.
- Click on the **Library** tab.
    - Uncheck any selected libraries.
- Click on the **Modifications** tab.
    - Click on the **Edit list** button next to **Structural modifications**.
    - In the **Edit Structural Modifications** form, remove any modifications in the **Modifications** list by clicking on the modification name and clicking on the **Remove** button.
    - Click the **Add** button in the **Edit Structural Modifications** form.
    - Choose “Carbamidomethyl (C)” from the **Name** dropdown list in the **Edit Structural Modification** form.
    - Click the **OK** button in the **Edit Structural Modification** form.
    - Click the **OK** button in the **Edit Structural Modifications** form.
    - Check the “Carbamidomethyl (C)” modification in the **Structural modifications** list.
    - Uncheck any selected modifications in the **Isotope modifications** list.
- Click the **OK** button in the **Peptide Settings** form.

Configure the transition settings as follows:

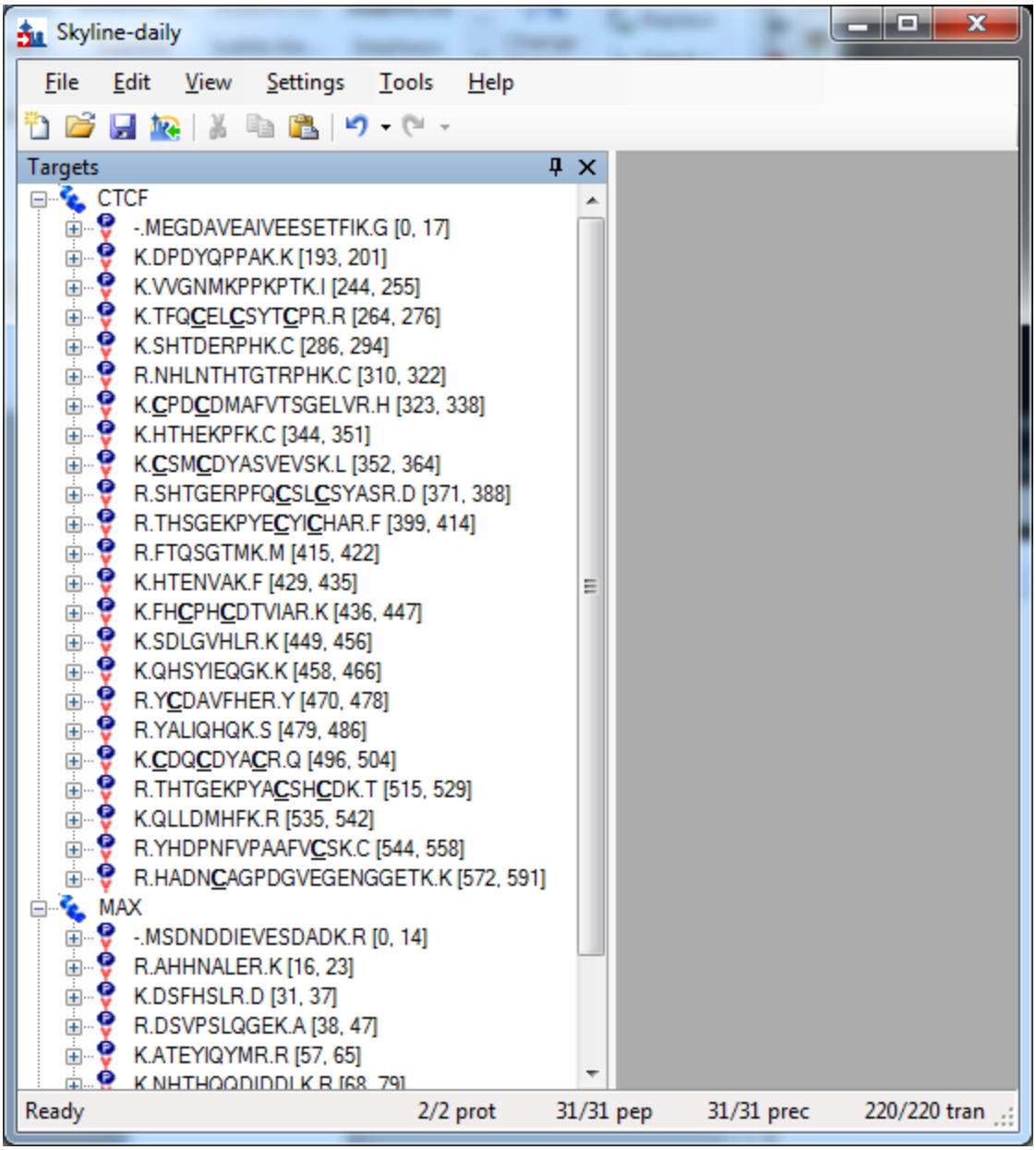
- Click on **Transition Settings** in the **Settings** menu.
- Click on the **Filter** tab.
    - Enter ‘2’ in the **Precursor charges** textbox.
    - Enter ‘1’ in the **Ion charges** textbox.
    - Enter ‘y’ in the **Ion types** textbox.
    - Select “ion 3” from the Product ions **From** dropdown list.
    - Select “last ion – 1” from the Product ions **To** dropdown list.
    - Uncheck any selected options under **Always add**.
    - Make sure that the **Precursor m/z exclusion widow** textbox is blank.

    - Check **Auto-select all matching transitions**.
- Click on the **Library** tab.
    - Uncheck **If a library spectrum is available, pick its most intense ions**
- Click on the **Instrument** tab.
    - Enter 50 in the **Min m/z** textbox.
    - Enter 1500 in the **Max m/z** textbox.
    - Uncheck **Dynamic min product m/z**.
- Click the **OK** button in the **Transition Settings** form.

The files you downloaded for this tutorial include the file 'CTCF_MAX.txt'. This file contains sequences for the proteins CTCF and MAX. To add these proteins to the new Skyline document do the following:

- From the **File** menu select **Import** and click on **FASTA**.
- Browse to and select 'CTCF_MAX.txt' in the file browser.
- Click the **Open** button.

The Skyline window should now look like this:

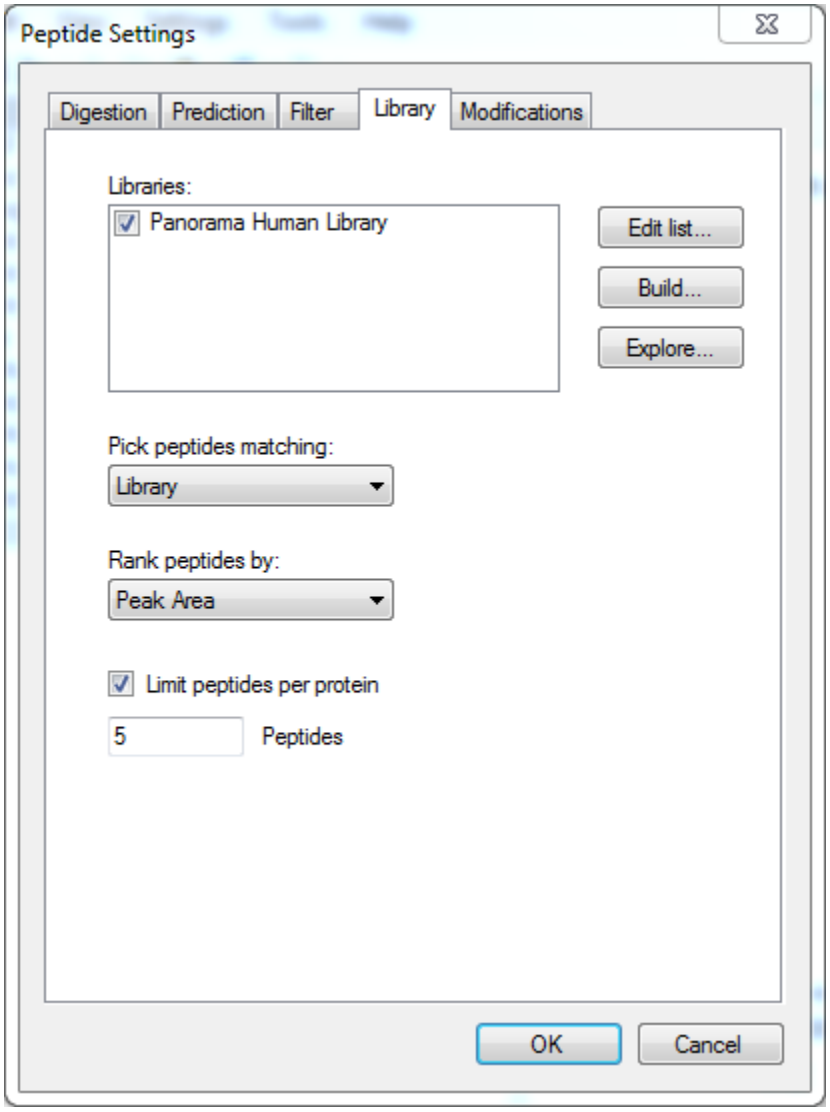


The settings you configured in Skyline have resulted in 31 peptides for the two proteins. If you export a transition list for this document without applying any filtering you would be measuring 220 transitions. However, you can take advantage of the information contained in the chromatogram library to limit your measurements to the best responding precursors and product ions for the two proteins. Follow these steps to filter the list of peptides and transitions:

- Click on **Peptide Settings** in the **Settings** menu.
- Click on the **Library** tab.
- Click on the **Edit List** button.

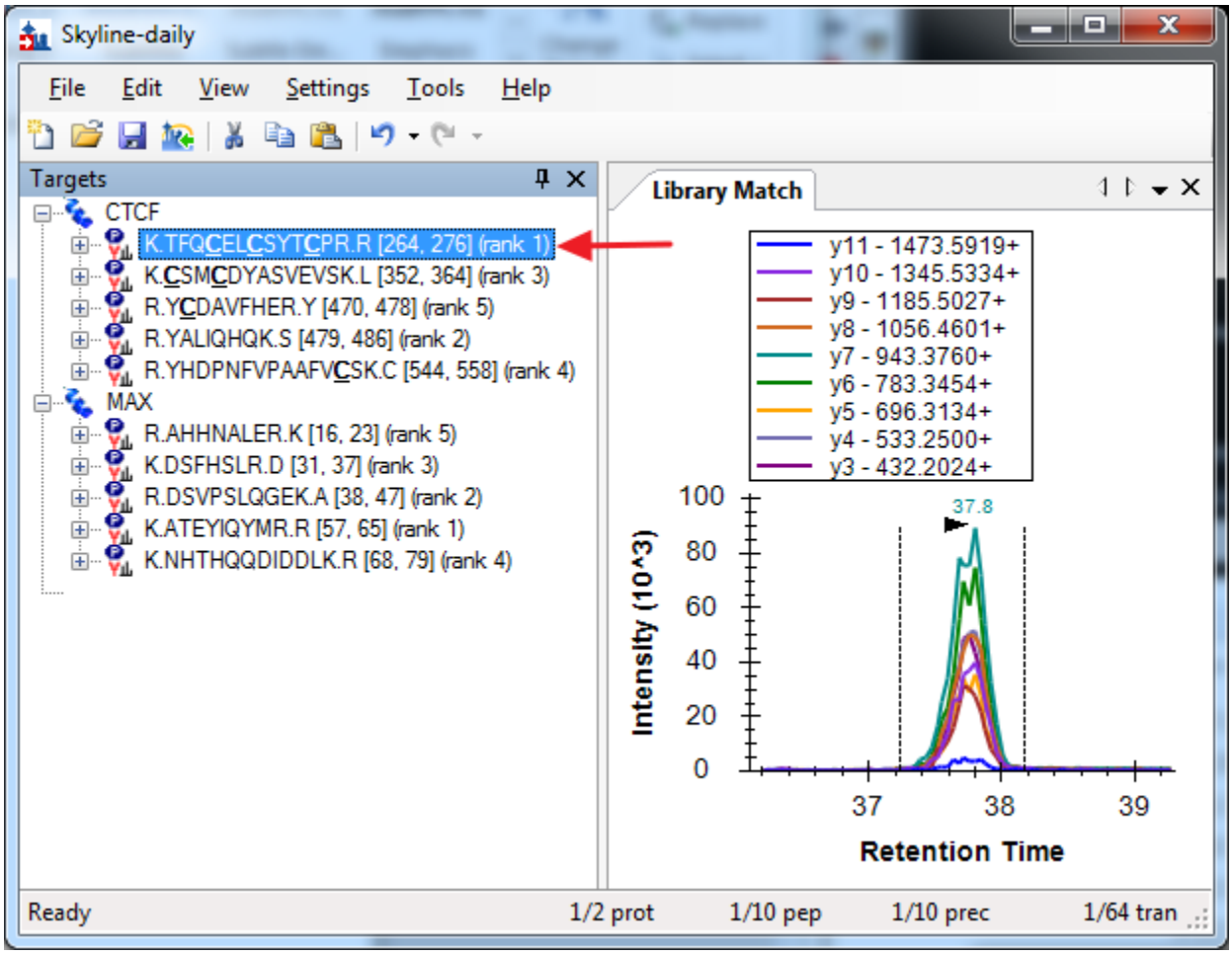
- In the **Edit Libraries** form click on the **Add** button.
- Enter 'Panorama Human Library' in the **Name** field of the **Edit Library** form.
- Click on the **Browse** button and select the chromatogram library file (Human_rev3.clib) that you downloaded earlier.
- Click on the **OK** button in the **Edit Library** form.
- Click on the **OK** button in the **Edit Libraries** form.
- In the **Library** tab of the **Peptide Settings** form, check the box next to 'Panorama Human Library'.
- Select **Library** from the **Pick peptides matching** dropdown menu.
- Select **Peak Area** from the **Rank peptides by** dropdown menu.
- Check the **Limit peptides per protein** box and enter 5 in the **Peptides** field.

The **Peptide Settings** form should now look as follows:



- Click on the **OK** button.
- Select the first peptide for the protein 'CTCF'.
- From the **View** menu, select **Library Match** to display the library chromatogram for the selected peptide in the **Library Match** tab.

The number of transitions in the document should have gone down from 220 to 64. Skyline also displays a rank for each peptide as indicated in the image below. This rank is based on the total peak area, summing all transition peak areas, for the peptide in the library.

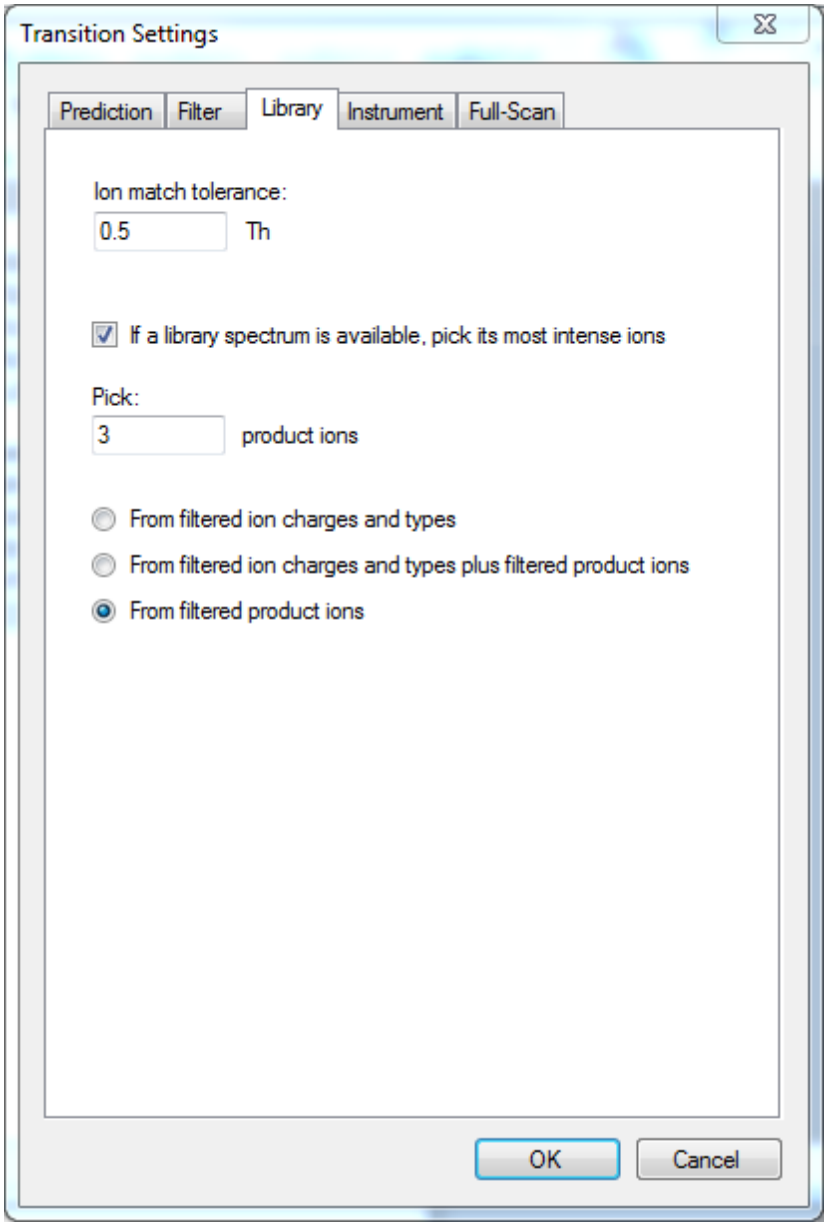


This number can be further reduced by limiting the transitions to the top ranking transitions in the library.

- Click on **Transition Settings** in the **Settings** menu.
- Click on the **Library** tab.
- Check the box next to **If a library spectrum is available, pick its most intense ions**.
- Enter 3 in the **Pick product ions** textbox.

- Select **From filtered product ions.**

The **Transition Settings** form should look like this:



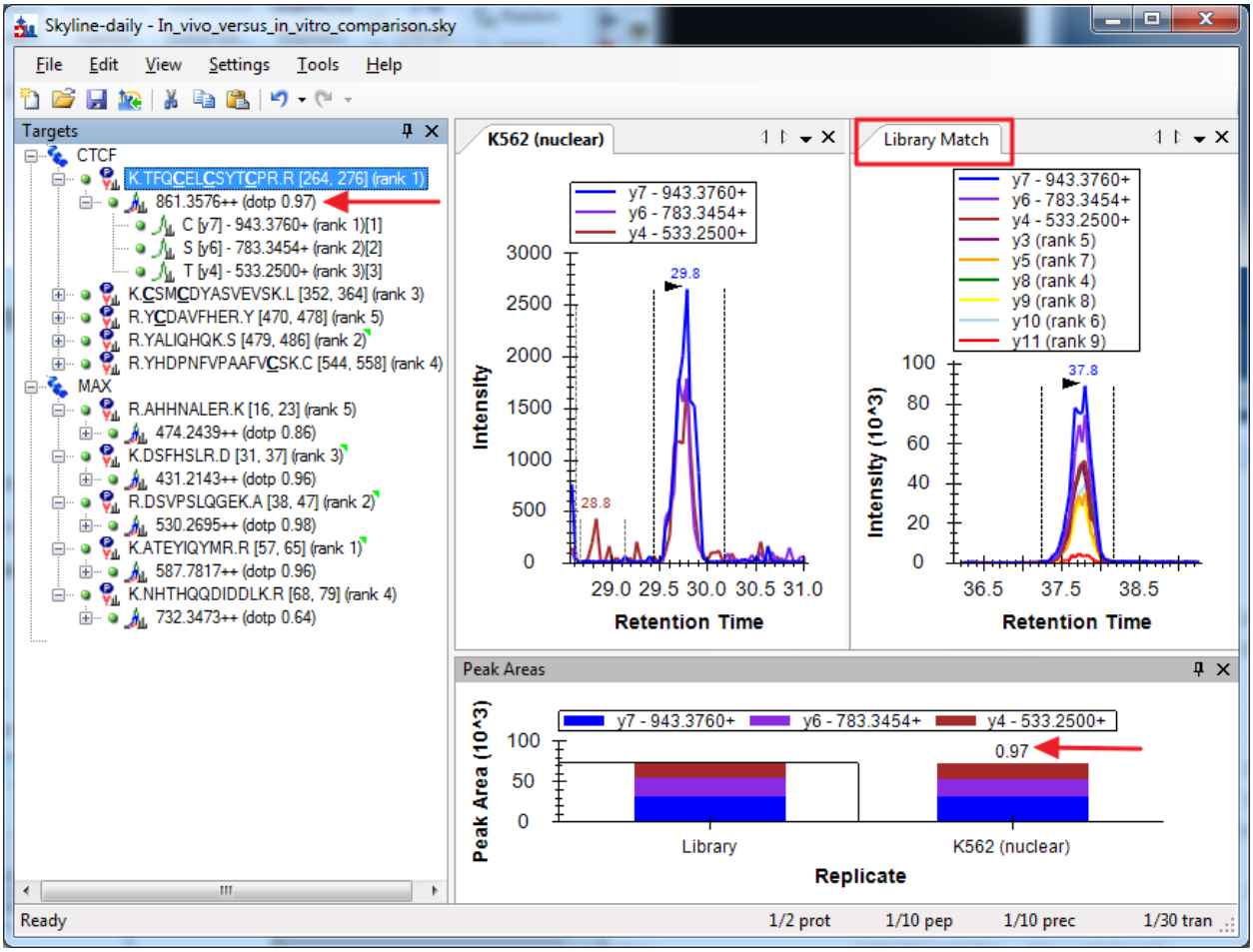
- Click on the **OK** button.

The number of transitions in this document should now be 30.

You can now export a transition list or a native instrument method for this document, and acquire data. Skyline offers the ability to export transition lists and SRM methods for instruments from several different instrument manufacturers. Select **Export** from the **File** menu and click on either **Transition List** or **Method** to view the list of supported instrument types in the **Instrument type** dropdown menu.

Please see the Targeted Method Editing and Targeted Method Refinement Skyline tutorials for more information on exporting transition lists and SRM Methods.

The files you downloaded for this tutorial already contain a document with data acquired on a triple quadrupole mass spectrometer. Open the file "In_vivo_versus_in_vitro_comparison.sky" in Skyline. The Skyline window should look like this:



Click on the peptide **TFQCELCSYTCPR.** You should see the chromatogram for this peptide from the newly acquired data side-by-side with the chromatogram from the Human_rev3.clib library. The tab titled **K562 (nuclear)** has the chromatogram from the new data, and the tab titled **Library Match** displays the library chromatogram.

If you do not see the **Library Match** tab you can display it by clicking on **Library Match** in the **View** menu. Skyline also calculates a dot product as a measure of similarity between the new data and the library chromatogram. This is displayed next to the precursor, as marked in the image above. For the

peptide **TFQCELCSYTCPR** the similarity is high with a dot product of 0.97, and that gives a measure of confidence in the peptide identification.

Conclusion

In this tutorial, you have gone through the steps of creating a library folder in Panorama for your curated, targeted results. You have built a chromatogram library in Panorama, and used it in Skyline to select the peptides and product ions to measure in a new experimental setting. You have also compared chromatograms acquired in a new experiment with data contained in the library to validate peptide identifications.

Reference List

1. Stergachis, A., MacLean, B., Lee, K., Stamatoyannopoulos, J. A., & MacCoss, M. J., Rapid empirical discovery of optimal peptides for targeted proteomics *Nature Methods* **8**,1041–1043 (2011)